LYONDELL

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8EHQ-1104-15137

MR281345

VIA COURIER

November 23, 2004

US Environmental Protection Agency
EPA East Mall/Mail Code 7407M
1201 Constitution Avenue, NW
Washington DC 20004

Attention: TSCA 8(e) Coordinator

RE: **8EHQ Number: 8EHQ-02-15137 (Submission of Final Report)**
Pyrolysis C5 (Hydrocarbons, C5-rich; CASRN 68476-55-1)
Dose Range Finding Study in Rats by Inhalation Exposure

Dear Sir or Madam:

In April 2002, Lyondell Chemical Company (Lyondell) submitted information to EPA in accordance with Section 8(e) of the Toxic Substances Control Act (TSCA) and EPA's 1991 Section 8(e) Reporting Guide. In that correspondence, Lyondell reported health effects information resulting from a dose range finding study in rats by inhalation exposure to a Pyrolysis C5 stream, a hydrocarbon distillate fraction separated from pyrolysis gasoline that consists primarily of C5 dienes and low levels of higher boiling C4 substances and volatile C6 hydrocarbons. The Pyrolysis C5 stream encompasses the substance identified by CAS Registry Number 68476-55-1 (*Hydrocarbons, C5-rich*). This submission was assigned 8EHQ Number 8EHQ-02-15137. At that time, the final report for this study was not available. Lyondell has now received the final report and is hereby providing a copy to EPA.

The study, sponsored by the American Chemistry Council, was conducted at Huntington Life Sciences Ltd. pursuant to the American Chemistry Council Olefins Panel testing plan for the C5 Non-Cyclics Category under the High Production Volume Chemical Challenge Program.

Should you have any questions or require additional details, please do not hesitate to call me at 713-309-2136. I may also be reached by facsimile at 713-951-1574 or by e-mail at patrick.gibson@lyondell.com.

Sincerely,

Patrick L. Gibson
Product Safety Specialist - Regulatory
Corporate TSCA Coordinator
Lyondell Chemical Company

Enclosure

DHL

EXP

Parcels: 1/1

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PYROLYSIS C5

DOSE RANGE FINDING STUDY IN RATS

BY INHALATION EXPOSURE

ACC Reference Number: OLF-63.0-HPV3-HLS

Sponsor

American Chemistry Council
1300 Wilson Boulevard
Arlington
VA 22209
USA

Research Laboratory

Huntingdon Life Sciences Ltd
Woolley Road
Alconbury
Huntingdon
Cambridgeshire

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APPENDICES

AUTHOR'S SIGNATURE PAGE

The study described in this report generally followed Good Laboratory Practice principles, however, due to the range-finding nature of this testing, no specific study related Quality Assurance procedures were performed and the report may not contain all the elements required by GLP.

A retention sample of the test substance was not taken nor held as it was not indefinitely stable and posed a safety hazard.

Amanda Brooke ..... 6 October 2004

Amanda J. Brooker, B.Sc. (Hons.), M.Sc., C.Biol., M.I.Biol., Date
Study Director,
Huntingdon Life Sciences Ltd.

This final report was accepted on the behalf of the Sponsor:

..... 9/29/04

Elizabeth J. Moran, Date
Sponsor's Representative.

CONTRIBUTING SCIENTISTS

STUDY MANAGEMENT

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TOXICOLOGY

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AEROSOL TECHNOLOGY

Ian S. Gilkison, M.A., Ph.D.,
Section Head, Aerosol Technology and Analysis Section,
Inhalation Studies Group.

SUMMARY

This study was performed to assess the effect of the test substance, Pyrolysis C5, on pregnant female rats to establish suitable dosages for a 4-week general toxicity and reproductive/developmental toxicity screening study.

Three groups, each of 6 time-mated female Crl:CD® rats, were exposed to a test atmosphere containing vapour of Pyrolysis C5, 6 hours a day, from Day 12 of pregnancy, using a whole body exposure system. Animals of Group 2 were exposed at a target concentration of 1000 ppm from Days 12-19 of pregnancy, inclusive. Due to the severity of effects in Group 3, exposures at a target concentration of 3000 ppm were terminated after Day 16 of pregnancy, and the rats allowed to recover prior to sacrifice on Day 20 of pregnancy. Similarly for Group 4, exposures at a target concentration of 5000 ppm were ended following 2 days and the animals sacrificed on Day 14 of pregnancy. A fourth group, Group 1, also of 6 females, acted as a control and was exposed to air only.

During the study, clinical signs, bodyweight and food consumption were recorded. Animals that died or were sacrificed prior to Day 20 of gestation were subjected to a full macroscopic examination, the uterine contents examined and the number of implantation sites recorded. On Day 20 of pregnancy the remaining animals were killed, examined macroscopically, and the uterus excised for examination of litter parameters.

The following comments in relation to principal findings are made in summary:

Achieved concentration

The chamber mean analysed concentration over the duration of the study were 0 (Control), 983, 2970 and 5080 ppm in Groups 1 to 4 respectively. Daily mean chamber levels for each chamber were calculated for measured chemical concentrations 12 times during each exposure day.

High dose group

Animals exposed to 5080 ppm showed a severe response over the first 2 days of exposure (Days 12 and 13 of pregnancy) that resulted in this group being terminated on Day 14 of pregnancy. There was a marked bodyweight loss and negligible food and water intake. During the 6-hour exposure, the animals were less responsive to external stimuli and had hunched posture. Following exposure the animals were hypersensitive to touch, had hunched posture and brown staining on the head and snout. On the morning following the second exposure (Day 14 of pregnancy) one animal died following a convulsive episode and the remaining animals were sacrificed.

At necropsy, congested lungs were apparent.

The vapour concentration of Pyrolysis C5 was estimated based on measured isoprene concentrations.

Intermediate dose group

In animals exposed to 2970 ppm a marked response to exposure were noted over the first 5 days of exposure (Days 12 to 16 of pregnancy inclusive) that resulted in treatment being withdrawn and animals remained untreated until Day 20 of pregnancy. During exposure, the animals exhibited hunched posture on 2 occasions, and showed signs post exposure of brown staining on the fur. There was a marked bodyweight loss between Days 12 and 15 of pregnancy, and food consumption was reduced. One animal showed excessive weight loss and was sacrificed following the exposures on Day 16. Recovery was apparent amongst the remaining females, with bodyweight gains and good food intake being recorded.

At necropsy congested lungs were apparent. There was no indication of any effect upon survival of the fetuses following exposure of the dams.

Low dose group

Animals exposed to 983 ppm tolerated the exposures from Days 12 to 19 of pregnancy well. The only effect was a transient slightly lower bodyweight gain and food intake during the first 4 days of exposure. There was no effect upon survival or growth of the fetuses to Day 20 *post coitum*.

Conclusion

It was concluded that pregnant females would tolerate exposures up to 1000 ppm and that this concentration would be suitable as the high exposure level in the subsequent OECD 422 study.

INTRODUCTION

The purpose of this study performed at Huntingdon Life Sciences Limited, Huntingdon, England was to assess the influence of the test substance on the pregnant female following exposure of the rats to repeated whole body inhalation administration of the test substance Pyrolysis C5, for 6 hours a day, from Day 12-19 after mating.

The study was performed to determine suitable dosages for a main 4-week general toxicity and reproductive/development screening study.

The study was not designed to meet any specific regulations or guidelines.

The test substance was administered by inhalation, a possible route for accidental or occupational exposure in man. The rat was the species of choice due to the requirement for a rodent species by regulatory agencies. The strain was selected on account of the availability of background data, relating to clinical and pathological parameters, at our laboratories.

RELEVANT STUDY DATES

Approved by:

Study Director:	19 February 2002
HRC Management:	19 February 2002
Study Sponsor:	26 February 2002
Animals arrived at HRC:	22 February 2002
Exposures commenced:	4 March 2002
Terminal kill:	12 March 2002
Experimental completion date:	12 March 2002

TEST SUBSTANCE

Identity:	Pyrolysis C5
CAS number:	68476-55-1, hydrocarbons, C5-rich
Alternative CAS numbers:	68476-43-7, 68603-00-9, 68956-55-8 and 68527-19-5
Alternative name:	C5, noncyclics
Composition:	Complex mixture of hydrocarbons (Appendix 7)
Lot number:	QA1001A100
Stability:	The expiry date of the test substance was not provided by the Sponsor. However, the test substance was analysed in an investigation, conducted as part of HLS Study Number CSS/017. The three major components analysed were shown to be stable for the duration of testing in this study (see Appendix 8 for Certificate of Analysis).
Appearance:	Clear, colourless liquid
Storage conditions:	Cool, dry well-ventilated area in the dark under nitrogen
Source:	Dow Chemical Company Louisiana Division Louisiana Highway PO Box 150 Plaquemine LA 70765-0150 USA
Date received:	17 January 2002

EXPERIMENTAL PROCEDURE

ANIMAL MANAGEMENT

A total of twenty six sexually mature female rats, approximately 9-10 weeks of age, of the Crl:CD®BR strain, which were time-mated to males of the same strain, were obtained from Charles River (UK) Ltd, Manston Road, Margate, Kent, England. The day of mating was considered Day 0 of pregnancy.

For those animals selected for the study, their estimated age at the start of treatment was 10-11 weeks and their bodyweights were in the range 280 g to 351 g.

The latest Health Screen Report published by the animal supplier was provided to HLS. In addition, the additional consignments of animals included a health screen relating to the current status of the breeding colony. These documents were sent to Huntingdon Life Sciences Veterinary Services immediately upon receipt for review and subsequent archiving.

Animals were assigned to groups randomly on arrival. From each delivery box, in no selective order, animals were allocated to labelled cages, commencing with the first cage, then the second and so on until three animals had been placed in each cage. A review of the mating details provided by the animal supplier confirmed that no females allocated to the same group had been mated with the same male.

The rats were housed in suspended stainless steel cages fitted with wire mesh tops and floors so that each cage contained 3 animals of the same sex. Plastic trays lined with absorbent paper were placed below each cage to collect animal excreta and the paper was changed daily. The rats were kept in a single room and cages from each group were positioned on an individual cage battery. The batteries holding each group of rats were housed in separate ventilation cabinets within the animal room. For the daily exposures, the animals were transferred to exposure chambers where they were housed in individual animal exposure cages of the suspended basket type, constructed of stainless steel mesh.

Animal room temperature and relative humidity controls were set at 19-25°C and 40-70% respectively. The actual recorded ranges were 18 to 21°C and 22 to 58% for temperature and relative humidity respectively. Permanent weekly recordings of these parameters were made using a Kent Clearspan recorder and these are archived with all other raw data for this study. Artificial lighting was controlled to give 12 hours light (0600 - 1800 hours) and 12 hours dark per 24 hours.

Animals had no access to food or water during each 6-hour exposure. At all other times, all rats had free access to tap water and pelleted UAR VRF1 Certified Diet. There was no information available to the Study Director to indicate that any non-nutrient substance likely to influence the effect of the test compound could reasonably be expected to be present in the diet or the drinking water, both of which were routinely subjected to regular chemical analyses. The results of these analyses are lodged in the Huntingdon Life Sciences Archives.

Throughout the study the animals were housed in the Department of Inhalation Toxicology, Building Y14, Room 010. The animals arrived on Day 2 of gestation. There was an acclimatisation period of 9 days before the start of exposures. The spare animals were retained during this period to replace any rat that showed signs of ill health. These spare rats were discarded once treatment had commenced with no further investigations performed.

ANIMAL IDENTIFICATION

Group	Rat numbers
1 (Air control)	1-6
2 (Low dose)	7-12
3 (Inter dose)	13-18
4 (High dose)	19-24

Each cage was identified by a coloured label according to group, and each label was uniquely numbered with the cage and study number. The animal number was tattooed on the tail of each animal in the cage.

ADMINISTRATION

The test substance, Pyrolysis C5, a colourless liquid, was administered at room temperature to rats by inhalation of a vapour for 6 hours a day using a whole body exposure system (0.75 m^3 exposure chambers). The control animals received air alone.

Group/colour code	Target exposure concentration (ppm)
1: White	0
2: Yellow	1000
3: Blue	3000
4: Pink	5000

The animals were exposed for a maximum of 8 consecutive days (Days 12 to 19 inclusive of pregnancy). The animals were exposed at approximately the same time each day and a separate exposure chamber was used for each group. Group 3 animals were exposed for 5 consecutive days (Days 12 to 16 inclusive of pregnancy) and Group 4 animals for 2 consecutive days (Days 12 and 13 of pregnancy).

Details of the exposure system, generation and sampling of the test atmospheres together with the results obtained are presented in the inhalation exposure and analysis section of this report (**ADMINISTRATION OF PYROLYSIS C5 BY INHALATION TO RATS**).

OBSERVATIONS AND MEASUREMENTS

Dated and signed records of all activities relating to the day-to-day running and maintenance of the study, as well as to the group observations and examinations outlined in this procedure were recorded in the Study Daybook. In addition, observations relating to individual animals made throughout the study were recorded.

Clinical signs

Individual animals were observed at least twice a day for any signs of behavioural changes, reaction to treatment or ill health. In addition, detailed observations were made daily, on the days of exposure, as follows:

1. Pre exposure observations.
2. Observations during exposure.
3. Observations within ½ to 1 hour of return to home cage.

During the daily exposure, obvious signs were recorded as a group response. Due to the type of exposure system used, the ability to observe individual animals during the exposures was severely restricted.

Dated and signed records of appearance, change and disappearance of clinical signs were maintained for individual animals.

Throughout the study, checks were made early in the working day (approximately 0800) and again in the afternoon approximately (1600) to look for dead and moribund animals.

Mortality

Animals that died or were sacrificed before Day 20 of gestation were subjected to a full macroscopic necropsy examination, the uterine contents examined and the number of implantation sites recorded.

Maternal bodyweight

The weight of each rat was recorded on Days 2, 5, 8, 12, 16 and 20 after mating. During the exposure period, bodyweights were recorded before the daily exposure. All animals were weighed at necropsy.

Maternal food consumption

The quantity of food consumed by each cage of rats was recorded for the periods of Days 2-4, 5-7, 8-11, 12-15 and 16-19 after mating.

TERMINAL STUDIES

Necropsy

All adult female animals were killed by carbon dioxide asphyxiation on Day 20 of gestation. Immediately prior to necropsy the animals were weighed.

A macroscopic examination of all rats was performed according to the following detailed necropsy procedure.

The abdomen of each animal was dissected and examined for congenital abnormalities and macroscopic pathological changes in the maternal organs. Abnormal tissues were preserved at the discretion of the pathologist. The ovaries and uteri were examined immediately to determine:

Number of corpora lutea
Number of implantation sites
Number of resorption sites (early, late and total)
Number and distribution of embryofoetal deaths
Individual foetal weight from which the litter weight was calculated
Individual placentae weights
Foetal abnormalities

Embryo-foetal deaths were classified as:

Early: only placenta visible at termination
Late: both placenta and embryonic remnants visible at termination

All foetuses were subjected to an external examination and the sex recorded. Each foetus was individually identified according to location within the litter. Following external examination all foetuses were humanely killed by cold plate euthanasia, then discarded.

TREATMENT OF DATA

Calculated values

For presentation purposes the values shown in appendices may be rounded. For calculation of group mean and derived individual values, unrounded values may have been used. Therefore it may not always be possible to calculate these values exactly by using data presented in the appendices.

Where appropriate data have been expressed as group means with standard deviations (SD).

Maternal bodyweight

Group mean values and SD were calculated for Days 2, 5, 8, 12, 16 and 20 of gestation for females with live young at Day 20. Weight changes were also calculated and plotted graphically with respect to Day 12 of gestation.

Maternal food consumption

Group mean values and SD were calculated for 2-4, 5-7, 8-11, 12-15 and 16-19 of gestation for females with live young at Day 20.

Litter responses

Litter data group mean values and SD (where appropriate) were calculated for numbers of corpora lutea, implantations, resorptions (early, late and total) and live young (male, female and total) at Day 20 of gestation. The group mean sex ratio (percentage of males) was also calculated.

Pre-natal losses were considered separately for the pre- and post-implantation phases.

a) Pre-implantation loss

Pre-implantation loss was calculated from the formula:

$$\frac{\text{(Number of corpora lutea - Number of implantations)}}{\text{Number of corpora lutea}} \times 100$$

b) Post-implantation loss

Post-implantation loss was calculated from the formula:

$$\frac{\text{(Number of implantations - Number of live fetuses)}}{\text{Number of implantations}} \times 100$$

Group values were calculated using litter mean values. The number of implantations was substituted for the corpora lutea count in calculating pre-implantation loss where the number of implantations exceeded the corpora lutea count.

Group mean fetal, litter and placental weights

Group mean fetal and placental weights and SD were calculated for each group as:

$$\frac{\text{Total of individual litter mean fetal or placental weights}}{\text{Number of litters}}$$

Mean fetal weights and SD were also calculated for each sex.

Group mean litter weights and SD were calculated for each group as:

$$\frac{\text{Total of individual litter weights}}{\text{Number of litters}}$$

STATISTICAL ANALYSIS

The small sample size precluded meaningful statistical evaluation, apart from standard deviations. Inter-group differences were assessed by reference to control data.

ARCHIVING

All raw data generated by the Sponsor has been retained in the Sponsor's archives.

All specimens, raw data and study-related documents generated during the course of the study at Huntingdon Life Sciences, together with a copy of the final report were lodged in Huntingdon Life Sciences, Archives.

Such specimens and records will be retained for a minimum period of ten years, from the date of issue of the final report. At the end of the ten-year retention period the Sponsor will be contacted and advice sought on the future requirements. Under no circumstances will any item be discarded without the Sponsor's knowledge.

DEVIATION FROM PROTOCOL

Due to the termination of Group 4 animals, they were weighed on Day 14 and food consumption presented over the period Days 12-13 of pregnancy.

On occasions, lower temperature or relative humidity values were recorded. In isolation, there are considered not to have affected the integrity of the study.

The sex ratio was presented in addition to protocol requested data handling.

A GLP compliance statement is not presented in the report, at deviation with the protocol. The study is not claiming GLP compliance.

These changes are not considered to affect the integrity of the study.

RESULTS

CHAMBER ATMOSPHERE CONDITIONS

Chamber analysed concentration of Pyrolysis C5

The data are presented in **ADMINISTRATION OF PYROLYSIS C5 BY INHALATION TO RATS** appended to this report.

The data are summarised below:

Group	Study mean concentration ppm	
	Target	Analysed
2 (Low dose	1000	983
3 (Inter dose)	3000	2970
4 (High dose)	5000	5080

The analysed concentrations were in agreement with the target concentrations.

CLINICAL OBSERVATIONS

ADULT FEMALES (Figure 1, Tables 1-3, Appendices 1-4)

High dose group

Group 4 animals were observed to be less responsive to external stimuli and show hunched posture during exposures. On the morning following the first and second exposure they were sensitive to touch, had hunched posture and brown staining on the head/snout. A marked weight loss (mean of 46 g; 15% of total bodyweight) was evident over the first two days of treatment and there was negligible food intake. Water intake appeared to be reduced on visual inspection. Prior to the third exposure, one animal had a convulsive episode noted on handling, and subsequently died.

All remaining animals were not exposed on Day 14 of gestation (Day 3 of exposures) and since one animal had shown convulsions an died, in conjunction with 15% loss of bodyweight, the decision was made by the Study Director and named animals care welfare officer to humanely sacrifice the remaining animals. The animals were sent for *post mortem* examination.

Macroscopic changes for adult females included congested lungs, and staining of the fur. The fetuses appeared to be alive with no marked *in utero* losses.

Intermediate dose group

Clinical signs during exposure included hunched posture on Days 14-16 (3rd - 5th exposure). Staining of the fur was apparent pre and post exposures from Day 14 of gestation (3rd exposure).

Due to a marked weight loss (mean of 38 g; 12% of total bodyweight) over the first 4 days of treatment, associated with markedly lower food intake, treatment was discontinued following Day 16 and the animals allowed to recover. Bodyweight gain and good food intake was subsequently shown.

On Day 16 of gestation one intermediate dose animal (Animal no. 14) was sacrificed due to the extent of bodyweight loss (51 g; 16% of total bodyweight). Macroscopic examination revealed stained fur and minimally congested lungs. The fetuses were all alive and there was no evidence of *in utero* losses.

The remaining animals were sacrificed on Day 20 of gestation. Apart from congested lungs in some females, no treatment related macroscopic changes were apparent.

Low dose group

There were no clinical signs apparent amongst low dose animals.

Group mean bodyweight gain of the animals was slightly lower than the controls (6% for Group 2 compared to 11% for the controls) between Days 12 and 16 of gestation. Thereafter bodyweight gain was comparable with the controls. Food consumption was slightly lower than the controls, reflecting the pattern of bodyweight gain.

No treatment related macroscopic changes were noted at necropsy.

LITTER VALUES AND EXTERNAL FOETAL EXAMINATION AT DAY 20 POST COITUM
(Tables 4 and 5, Appendices 5 and 6)

In Groups 1 to 3 respectively all females sent for necropsy at Day 20 of gestation had live young for assessment, providing 6, 6 and 5 litters and 76, 81 and 67 fetuses respectively.

Implantations and litter data

There were no treatment-related effects on the number of implantations or subsequent litter size. Implantation losses were low and there was no evidence of the selective loss of either sex, as evidenced by a similar sex ratio in all groups.

Fetal, placental and litter weights

Fetal, litter and placental weights were unaffected by treatment in animals of the low dose group. In females where treatment had only been confirmed on Days 12 to 16 of gestation litter, fetal and placental weight was lower than the control. It is uncertain whether this would be due to the test substance perse but could be more likely to reflect the marked weight loss apparent in the pregnant animal.

Macroscopic foetal examination

There were no gross external macroscopic changes detected in the fetuses.

DISCUSSION AND CONCLUSION

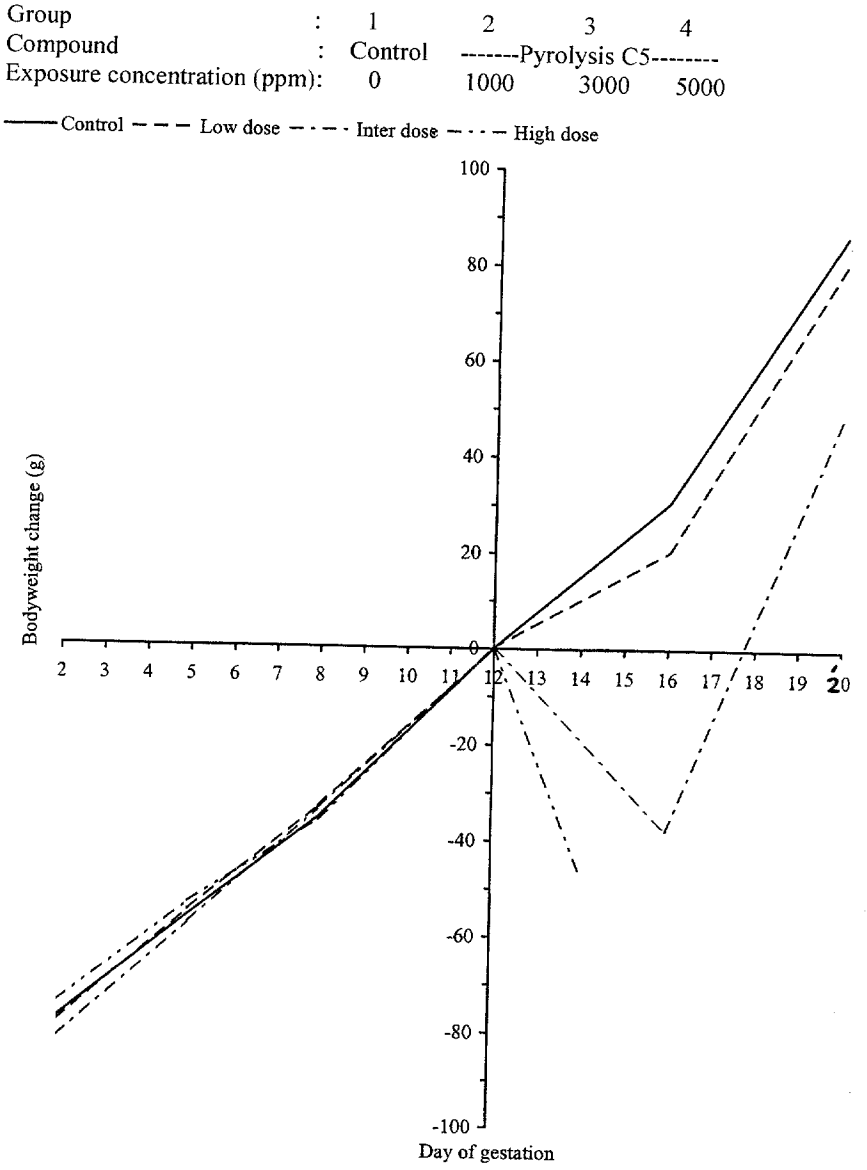
Within the context of this study, exposure of Pyrolysis C5 to female rats from Day 12 of gestation was not tolerated at concentrations of 3000 or 5000 ppm. The animals showed markedly lower food intake and bodyweight losses. At 5000 ppm the condition of the animals, including one animals that died, necessitated sacrifice of this group of animals. At 3000 ppm in the opinion of the Study Director and named animal welfare officer, it was decided that the animals may show signs of recovery following termination of exposures, but would not tolerate exposures beyond Day 15 of gestation.

Amongst animals receiving 1000 ppm, the exposures were well tolerated with the only finding being a transient slightly lower bodyweight gain and food intake over the first 4 days of treatment. There were no effects on the specific litter parameters investigated.

It was concluded that animals would tolerate exposures up to 1000 ppm in the subsequent OECD 422 study in the rat.

FIGURE 1

Bodyweight change - group mean values (g)



Treatment commenced Day 12
Group 4 females sent for necropsy on Day 14 of gestation

TABLE 1

Clinical observations during exposure - group incidence summary - adult females

Group : 1 2 3 4
Compound : Control -------Pyrolysis C5--------
Exposure concentration (ppm): 0 1000 3000 5000

Group	Signs	Day of gestation														
		12		13			14			15			16			
		Time in hours		Time in hours			Time in hours			Time in hours			Time in hours			
		0.5	6	0.5	1	6	0	3	6	0	5	6	0	5	6	
3	Brown staining around muzzle						✔		✔	✔		✔	✔		✔	
	Hunched posture							✔	✔		✔	✔		✔	✔	
4	Less responsive to external stimuli	✔	✔	✔		✔										
	Brown staining around muzzle			✔		✔										
	Hunched posture				✔	✔										

Only groups with clinical signs are listed
Treatment commenced Day 12 of gestation, treatment withdrawn following Day 16 of gestation for Group 3 and 13 of gestation for Group 4

TABLE 2

Bodyweight and bodyweight change - group mean values - adult females

Group	:	1	2	3	4
Compound	:	Control	-------Pyrolysis C5--------		
Exposure concentration (ppm):		0	1000	3000	5000

Group		Bodyweight (g) on Day of gestation					
		2	5	8	12	16	20
1	Mean	230	251	272	307	338	394
	SD	16	17	20	21	25	28
	n	6	6	6	6	6	6
2	Mean	235	258	280	314	334	394
	SD	15	12	14	16	18	23
	n	6	6	6	6	6	6
3[b]	Mean	238	262	286	320	283	369
	SD	17	18	18	21	26	33
	n	6	6	6	6	6	5
4[b]	Mean	238	259	276	312	267[a]	
	SD	18	14	15	18	15	
	n	6	6	6	6	6	

SD Standard deviation

Group		Bodyweight change (g) relative to start of treatment on Day of gestation					
		2	5	8	12	16	20
1	Mean	-78	-56	-36	0	31	87
2	Mean	-79	-55	-33	0	21	80
3[b]	Mean	-82	-58	-34	0	-38	49
4	Mean	-75	-54	-36	0	-46[a]	

Treatment commenced Day 12

[a] Day 14 of gestation as Group 4 females were sent for necropsy

[b] Treatment was withdrawn after day 16 of gestation

TABLE 3

Food consumption - group mean values (g/rat/day) - adult females

Group	:	1	2	3	4
Compound	:	Control	--------Pyrolysis C5--------		
Exposure concentration (ppm):		0	1000	3000	5000

Group		Food consumed during days of gestation (g/rat/day)				
		2-4	5-7	8-11	12-15	16-19
1	Mean	29	29	31	34	35
	SD	1	0	0	1	2
	n	2	2	2	2	2
2	Mean	29	30	32	29	33
	SD	2	1	1	1	0
	n	2	2	2	2	2
3[b]	Mean	31	31	33	13	27
	SD	3	3	3	1	5
	N	2	2	2	2	2
4	Mean	28	29	31	3[a]	
	SD	1	0	2	1	
	n	2	2	2	2	

SD Standard deviation

Treatment commenced Day 12

[a] Days 12-13 of gestation as Group 4 females were sent for necropsy on Day 14 of gestation

[b] Treatment was withdrawn after exposures or Day 16 of gestation

TABLE 4

Implantations and litter size - group mean values

Group : 1 2 3 4
Compound : Control -------Pyrolysis C5--------
Exposure concentration (ppm): 0 1000 3000 5000

Group		Corpora Lutea	Implantations	Resorptions			Live young			Sex ratio (% M)	Implantation loss (%)	
				Early	Late	Total	Male	Female	Total		Pre-	Post-
1	Mean	14.8	13.3	0.7	0.0	0.7	6.5	6.2	12.7	50.7	9.4	4.7
	SD	2.3	1.5				2.7	2.1	1.2			
	n	6	6	6	6	6	6	6	6	6	6	6
2	Mean	15.0	14.8	1.3	0.0	1.3	6.2	7.3	13.5	46.0	1.0	9.3
	SD	1.7	1.5				1.3	1.9	2.2			
	n	6	6	6	6	6	6	6	6	6	6	6
3[a]	Mean	15.0	14.2	0.6	0.2	0.8	6.2	7.2	13.4	44.2	6.7	6.1
	SD	2.6	2.6				2.9	1.9	3.0			
	n	5	5	5	5	5	5	5	5	5	5	5

SD Standard deviation
[a] Treatment was withdrawn after Day 16 of gestation

TABLE 5

Foetal, placental and litter weights - group mean values (g)

Group	:	1	2	3	4
Compound	:	Control	-------Pyrolysis C5--------		
Exposure concentration (ppm):		0	1000	3000	5000

Group		Placental weight (g)	Litter weight (g)	Fetal weights (g)		
				Males	Females	Overall
1	Mean	0.58	50.23	4.02	3.88	3.96
	SD	0.04	5.54	0.21	0.16	0.16
	n	6	6	6	6	6
2	Mean	0.57	52.90	4.01	3.83	3.91
	SD	0.04	9.51	0.21	0.17	0.19
	n	6	6	6	6	6
3[a]	Mean	0.44	47.96	3.73	3.50	3.60
	SD	0.06	9.39	0.16	0.20	0.15
	n	5	5	5	5	5

SD Standard deviation
n Number of litters
[a] Treatment was withdrawn after Day 16 of gestation

APPENDIX 1

Daily dose observations - individual findings - adult females

Group : 1 2 3 4
Compound : Control -------Pyrolysis C5--------
Exposure concentration (ppm): 0 1000 3000 5000

Group	Animal Number	Signs	Day of gestation															
			13		14		15		16		17		18		19		20	
			Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
3	13	Brown staining on snout			✔	✔	✔	✔	✔	✔	✔							
		Brown staining on head			✔	✔	✔	✔					✔					
		Hair loss dorsal				✔	✔	✔	✔	✔	✔		✔		✔		✔	
	14	Brown staining on snout		✔	✔	✔	✔	✔	✔									
		Brown staining on head				✔	✔	✔	✔									
		Yellow/brown staining urogenital area					✔	✔	✔						✔		✔	
	15	Brown staining on snout			✔	✔	✔	✔	✔	✔	✔							
		Brown staining on head				✔	✔	✔		✔	✔							
		Hair loss dorsal				✔	✔	✔	✔	✔	✔		✔					
	16	Brown staining on snout		✔	✔	✔	✔	✔	✔	✔	✔							
		Brown staining on head				✔												
	17	Brown staining on snout				✔	✔	✔										
		Brown staining on head				✔				✔								
	18	Brown staining on snout			✔	✔	✔	✔	✔	✔								
		Brown staining on head			✔	✔	✔	✔		✔	✔		✔					

Only animals with clinical signs are listed
Treatment commenced Day 12 of gestation, treatment withdrawn following exposure on Day 16

APPENDIX 1

(Daily dose observations - continued)

Group : 1 2 3 4
Compound : Control -------Pyrolysis C5--------
Exposure concentration (ppm): 0 1000 3000 5000

Group	Animal Number	Signs	Day 2 Pre	Day 2 Post	Day 3 Pre	Day 3 Post
4	19	Brown staining on snout			✔	
		Brown staining on head		✔	✔	
		Sensitive to touch			✔	
		Hunched posture			✔	
	20	Brown staining on snout			✔	
		Brown staining on head			✔	
		Sensitive to touch			✔	
		Hunched posture			✔	
	21	Brown staining on snout			✔	
		Brown staining on head			✔	
		Sensitive to touch			✔	
		Hunched posture			✔	
	22	Brown staining on snout		✔	✔	
		Brown staining on head			✔	
		Sensitive to touch			✔	
		Hunched posture			✔	
	23	Brown staining on snout		✔	✔	
		Brown staining on head		✔	✔	
		Sensitive to touch			✔	
		Hunched posture			✔	
	24	Brown staining on head		✔	✔	
		Sensitive to touch			✔	
		Unsteady gaint			✔	
		Hunched posture			✔	

Only animals with clinical signs are listed

APPENDIX 2

Clinical signs and necropsy - individual findings for adult females

Group	:	1	2	3	4
Compound	:	Control	-------Pyrolysis C5--------		
Exposure concentration (ppm):		0	1000	3000	5000

Group	Animal number	Clinical signs observed (Day after mating)	Necropsy findings (Day after mating)
Group 1 (Control)	4	-	Hair loss and encrustation on left side of head (D20)
		-	
		-	
Group 2 (Low)	8	-	A few scabs, 3 x 2 mm on left side of head. Hairloss dorsal abdomen. (D20)
		-	
Group 3 (Inter)	13	-	Hair loss dorsal abdomen (D20)
	14	Red/brown staining head and snout, yellow/brown staining urogenital area (D16)	Fur genital region, yellow stained face and forepaws red/brown stained. Lungs minimal congested. All pups appear normal and alive (D16)
	15	Hairloss dorsal (D20)	Hair loss on upper dorsal abdomen
		-	
Group 4 (High)	19	Red /brown staining head and snout. Sensitive to touch. Hunched posture (D14)	Lungs patchy, congestion minimal. Fur, face and forepaws red/brown stained. All pups appear normal and alive (D14)
	20	Red/brown staining snout. Sensitive to touch. Hunched posture (D14)	Lungs patchy, congestion moderate. Fur, face and forepaws red/brown stained. All pups appear alive and normal
	21	Animal died following convulsions. Red/brown staining head and snout (D14)	Fur – cranial region and perianal region red/brown stained. Lungs – congested, severe. Thymus congested. All pups appear alive and normal
	22	Brown staining head and snout. Sensitive to touch (D14)	Lungs patchy, congestion severe. Fur cranial region and perianal region red/brown stained. All foetuses appear normal and alive (D14)
	23	Red/brown staining head and snout. Sensitive to touch (D14)	Lungs patchy congestion severe. Fur cranial region and perianal region and forepaws red/brown stained. All foetuses appear normal and alive (D14)
	24	Red/brown staining snout. Yellow staining urogenital region (D14)	Lungs patchy congestion. Fur perianal region red/brown stained, genital region yellow stained. All foetuses appear normal and alive (D14)
		-	

\- No clinical signs evident

Only animals with findings are presented; all other animals had no abnormalities detected

APPENDIX 3

Bodyweights - individual values - adult females

Group : 1 2 3 4
Compound : Control -------Pyrolysis C5--------
Exposure concentration (ppm): 0 1000 3000 5000

Group	Animal number	Bodyweight on Day of gestation					
		2	5	8	12	16[b]	20
1	1	240	264	289	324	351	411
	2	250	271	288	325	362	421
	3	229	253	272	307	345	404
	4	213	230	249	282	305	360
	5	235	257	286	325	357	410
	6	210	231	246	280	307	356
2	7	228	254	282	319	341	406
	8	223	244	257	285	302	355
	9	236	264	285	318	338	402
	10	226	252	280	313	328	382
	11	264	279	301	333	356	420
	12	232	257	276	313	339	398
3	13	260	291	312	351	324	420
	14	242	260	283	319	268	[a]
	15	255	278	304	338	296	380
	16	228	252	275	302	247	336
	17	225	250	280	316	285	368
	18	219	243	264	295	275	343
4	19	248	262	283	313	264	[b]
	20	243	264	281	317	263	[b]
	21	233	256	270	307	260	[b]
	22	257	272	290	329	290	[b]
	23	205	232	248	280	247	[b]
	24	240	267	286	328	275	[b]

Treatment commenced Day 12
[a] Animal No. 14 sent for necropsy on Day 16 of gestation
[b] Group 4 females sent for necropsy on Day 14 of gestation

APPENDIX 4

Food consumption – cage mean values - adult females

Group	:	1	2	3	4
Compound	:	Control	-------Pyrolysis C5--------		
Exposure concentration (ppm):		0	1000	3000	5000

Group	Cage number	Food consumed during days of gestation (g/rat/day)				
		2-4	5-7	8-11	12-15	16-19
1	1	30	29	31	35	36
	2	28	28	31	33	33
2	3	28	29	31	28	33
	4	31	31	33	30	34
3	5	33	33	35	14[a]	24
	6	28	29	31	13	30
4	7	27	29	30	3[b]	
	8	29	29	32	4[b]	

Treatment commenced Day 12

[a] Only 2 animals left on Day 15 of gestation onwards as Animal No. 14 sent for necropsy

Food consumed from Day 12-13 gestation, Group 4 females sent for necropsy on Day 14 of gestation

APPENDIX 5

Implantations and litter size - individual values at Day 20

Group : 1 2 3 4
Compound : Control -------Pyrolysis C5--------
Exposure concentration (ppm): 0 1000 3000 5000

Group	Animal	Corpora lutea	Implants	Resorption			Live young			Sex ratio	Implantation loss (%)	
				Early	Late	Total	Male	Female	Total		Pre-	Post-
1	1	18	15	1	0	1	11	3	14	78.6	16.7	6.7
	2	17	14	0	0	0	8	6	14	57.1	17.6	0.0
	3	13	11	0	0	0	6	5	11	54.5	15.4	0.0
	4	12	12	0	0	0	4	8	12	33.3	0.0	0.0
	5	14	14	2	0	2	6	6	12	50.0	0.0	14.3
	6	15	14	1	0	1	4	9	13	30.8	6.7	7.1
2	7	15	15	2	0	2	6	7	13	46.2	0.0	13.3
	8	14	14	3	0	3	6	5	11	54.5	0.0	21.4
	9	17	16	1	0	1	8	7	15	53.3	5.9	6.3
	10	14	14	1	0	1	4	9	13	30.8	0.0	7.1
	11	17	17	0	0	0	7	10	17	41.2	0.0	0.0
	12	13	13	1	0	1	6	6	12	50.0	0.0	7.7
3	13	18	17	0	0	0	7	10	17	41.2	5.6	0.0
	15	14	15	0	0	0	8	7	15	53.3	0.0	0.0
	16	11	10	0	1	1	1	8	9	11.1	9.1	10.0
	17	16	15	2	0	2	8	5	13	61.5	6.3	13.3
	18	16	14	1	0	1	7	6	13	53.8	12.5	7.1

APPENDIX 6

Foetal, placental and litter weights - individual values at Day 20

Group	:	1	2	3	4
Compound	:	Control	-------Pyrolysis C5--------		
Exposure concentration (ppm):		0	1000	3000	5000

Group	Animal number	Placental weights(g)		Litter weight (g)	Fetal weights (g)					
					Males		Females		Total	
		Mean	SD		Mean	SD	Mean	SD	Mean	SD
1	1	0.63	0.06	58.95	4.25	0.09	4.06	0.25	4.21	0.15
	2	0.53	0.04	52.52	3.85	0.12	3.62	0.20	3.75	0.19
	3	0.57	0.07	43.88	4.15	0.17	3.80	0.15	3.99	0.24
	4	0.61	0.06	46.49	3.74	0.69	3.94	0.12	3.87	0.39
	5	0.57	0.03	46.82	3.91	0.27	3.90	0.19	3.90	0.22
	6	0.57	0.06	52.73	4.21	0.20	3.99	0.14	4.06	0.18
2	7	0.58	0.04	50.37	3.94	0.06	3.82	0.16	3.87	0.14
	8	0.51	0.04	40.02	3.71	0.32	3.55	0.09	3.64	0.25
	9	0.61	0.08	60.43	4.08	0.18	3.97	0.15	4.03	0.17
	10	0.59	0.13	49.35	3.91	0.24	3.75	0.25	3.80	0.25
	11	0.55	0.05	67.15	4.09	0.12	3.85	0.19	3.95	0.20
	12	0.60	0.06	50.10	4.33	0.14	4.02	0.18	4.18	0.22
3	13	0.50	0.04	59.46	3.63	0.20	3.40	0.18	3.50	0.22
	15	0.45	0.05	50.66	3.52	0.11	3.22	0.24	3.38	0.23
	16	0.50	0.04	33.37	3.94	[a]	3.68	0.23	3.71	0.23
	17	0.38	0.03	47.80	3.78	0.12	3.51	0.20	3.68	0.20
	18	0.39	0.03	48.51	3.76	0.14	3.70	0.24	3.73	0.18

[a] Only 1 male in litter

APPENDIX 7

Pre-shipment composition of the test substance[1]

Pyrolysis C5s

Component identity	*Composition*
1,4-Pentadiene	2.6402%
Butyne-2	0.9455%
Pentene-1	6.3361%
2-Methyl-butene-1	3.5895%
Isoprene	17.8389%
trans-Pentene-2	2.8318%
cis-Pentene-2	1.7760%
2-Methyl-butene-2	2.5095%
trans-1,3-Pentadiene	10.0028%
Cyclopentadiene	7.7000%
cis-1,3-Pentadiene	6.1220%
Cyclopentene	7.0142%
Cyclopentane	1.3326%
Butenes	0.0032%
1,3-Butadiene	0.0403%
n-Butane	0.0193%
trans-Butene-2	0.1625%
cis-Butene-2	0.4682%
1,2-Butadiene	0.3055%
3-Methyl-butene-1	0.5599%
Isopentane	8.0069%
3,3-Dimethyl, 1-butene	0.0714%
4-Methyl-1-pentene	0.1610%
2-Methylpentane	1.7335%
1,5-Hexadiene	0.1675%
3-Methylpentane	0.3081%
Hexane	0.0410%
Dicyclopentadiene	5.8744%
Ethylacetylene	0.0102%
n-Pentane	9.5163%
2,3-Dimethylbutane	0.1772%
3-Methyl-1-pentene	0.3278%

[1] Information supplied by the Dow Chemical Company

APPENDIX 8

Certificate of analysis for Pyrolysis C5

CERTIFICATE OF ANALYSIS

DEPARTMENT OF PRODUCT CHEMISTRY (HUNTINGDON)

HUNTINGDON LIFE SCIENCES

Test substance: Pyrolysis C5

Batch number: QA1001A100

Analysis dates: Initial analysis: 19 February 2002
Final analysis: 6 August 2002

Data obtained as part of study: CSS/017

Purity determined by GC area percent.

Purity (initial analysis):

n-pentane:	9.3%
isoprene:	16.5%
trans 1,3 pentadiene:	10.4%

Purity (final analysis):

n-pentane:	10.9%
isoprene:	19.0%
trans 1,3 pentadiene:	11.9%

D A Howes
David A Howes,
Study Director,
Huntingdon Life Sciences Ltd.

21 May 2004
Date

CSS 011/020072

ADMINISTRATION OF PYROLYSIS C5

BY INHALATION TO RATS

Author
Simon Moore

CONTENTS

TEST SUBSTANCE AND ADMINISTRATION

TEST SUBSTANCE

The test substance, Pyrolysis C5, is a volatile liquid mixture with the boiling points of the majority of components below approximately 50°C.

A consignment, comprising fifteen cylinders, each with a stated net content of 45 lbs (Lot number 86756), was received from The Dow Chemical Company on 21 January 2002. The test substance was stored securely in the original containers in a large water tank contained in a refrigerated store with the temperature regulated at approximately 10°C until it was transferred to the atmosphere generation system.

Information provided by the Supplier indicated that the test substance was stable for the intended duration of use on the study. The composition was defined during Huntingdon Life Science report number CSS017/022791. Information regarding the purity and stability of the test substance is the responsibility of the Supplier.

ADMINISTRATION

The test material was administered to the rats by inhalation in whole-body exposure chambers as described below:

The chamber atmospheres were produced by metering the liquid test substance into glass vapour generators through which dried air was passed at a group dependent flow rate ranging from 50 to 150 l/minute. The atmosphere produced by the generation system was (except for Group 4) further diluted with air to give a total flowrate of 150 l/minute and the final chamber concentrations of test aerosol.

The in-line airflow to the vapour generation apparatus was verified using a dry type gas meter during the preliminary phase of the study. During the study, the airflow to the atmosphere generation system was monitored throughout each of the exposures using calibrated in-line tapered tube gas flowmeters.

The settings of the test substance metering system required to obtain the target chamber concentrations were determined during preliminary generation trials without animals present and based on the gas chromatographic (GC) analysis of chamber atmosphere samples. Minor adjustments were made to the test material delivery rates in order to maintain chamber concentrations close to target.

Animals assigned to Group 1 (Air control) received an exposure to compressed air only, from the same source as used for the generation of the test atmospheres.

The duration of administration was a single 6-hour exposure, daily, for 8 days for Groups 1 and 2 (Control and Low doses), 5 days for Group 3 (Intermediate dose) and 2 days for Group 4 (High dose). Groups 3 and 4 (Intermediate and High doses) were prematurely shortened due to clinical signs.

The usage of Pyrolysis C5 was determined, for each day of treatment, for each test group.

TEST ATMOSPHERE GENERATION (Figure A)

The vapour for each of the test groups was supplied from individual reservoirs of liquid Pyrolysis C5 maintained at pressure. The top of each reservoir [a] was fitted with a central, "O" - ring sealed filler cap, a system to allow pressurisation and release of the helium head pressure and a safety pressure release valve set to operate at above the study operating pressure. The reservoirs were mounted on electronic load cells [b] and the weight of each reservoir and contents could be displayed continuously. Each load cell was set to read zero weight with the empty reservoir in place before the first occasion of filling with the test substance and the minimum permissible start weight for the study exposures was calculated. Except during filling, the Pyrolysis C5 in the reservoirs was maintained under a helium pressure of 5 psi for Group 2 (low dose) and 10 psi for Groups 3 and 4 (Intermediate and High doses).

The pressurised reservoirs supplied the test substance to all vapour generators which comprised a glass frit contained in a glass vessel. For each test group, the liquid delivery line to the glass vessel was fitted with a toggle valve to allow isolation of the test substance supply. Downstream of this, a particulate filter (stainless steel, 0.5 μm pore size [c]) was fitted, to protect the subsequent metering valve from any entrained particulate. The metering valve (Nupro S Series Needle Valve [c]) controlled the test substance delivery rate. Fluid passing through each metering valve was delivered onto the glass frit surface of the vaporiser, at ambient temperature. The air supply (50 to 150 l/minute) for the generator was first passed through a separate copper coil, at ambient temperature. The test substance vapour in air mixture was passed through fibre reinforced PVC tubing (10 mm internal diameter).

For all groups exposed to Pyrolysis C5, the vapour/air mixture produced in the vapour generators was passed into the base of the secondary dilution vessel. A further supply of clean and dry air was supplied to Groups 2 and 3 to ensure a total chamber airflow of approximately 150 l/minute. The air supply for Group 4 was provided solely by the vapour generation system.

Diluent air flow was measured using a tapered tube flow meter situated at the front of a purpose-built stainless steel trolley on which the secondary dilution vessel was mounted. Generation air was measured on a similar flowmeter mounted on the vapour generation trolley.

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[a] Newson Gale Ltd, 51 Norsey Road, Billericay, Essex, CM11 1BG, England

[b] Huntleigh Industrial Controls Ltd, Load Cell Division, Portman Moor Industrial Estate, East Moors, Cardiff, South Glamorgan, CF22 2HB

[c] Nupro Co, Willoughby, Ohio 44094, USA

The test atmosphere was then passed through flexible ducting to a tangential inlet mounted at the apex of the appropriate exposure chamber.

A schematic of the vapour generation system is presented in Figure A.

The control group was exposed using a similar system to that used for the test groups, but received compressed air only at a rate of approximately 150 l/minute.

The air supplied to the vapour generators and secondary dilution vessels was filtered to remove any residual particulate and was dried (dew point ~2°C).

EXPOSURE CHAMBERS (Figure B)

The exposure chambers were of stainless steel and glass construction and consisted of a cuboidal body fitted with a pyramidal base and top. The internal volume of each chamber was approximately 0.75 m^3. At the apex of the upper pyramidal figure was the tangentially mounted air duct. Immediately below this was a perforated canister, which ensured equal distribution of the test atmosphere within the chamber.

Access to the chamber was through the front of the box section *via* a hinged door with a glass panel and stainless steel frame. The door was sealed using moulded rubber sealing strip.

Exposure cages constructed of stainless steel mesh were suspended on a framework arranged on 4 levels. Each level is able to hold four cages, with each cage capable of housing 4 rats individually. This gave a potential animal exposure capacity of 64 rats. In this investigation, six animal compartments on either level 2 or 3 of the 4 level chamber were used at any one time.

Projecting through the rear wall of each chamber was one 0.25 inch diameter stainless steel tube protruding between levels 2 and 3 of the chamber. This was used for collection of chamber atmosphere samples. Spatial distribution studies were conducted during preliminary trials of this study.

The pyramidal base of each chamber was fitted with a 2-inch drain. The drain connected with a common drainage system *via* a ball valve.

A square tubular exhaust plenum, 3 inches in diameter and perforated along the ventral surface, was situated in the pyramidal base. This connected to the main extract system.

A wet and dry alcohol bulb thermohygrometer was suspended in the chamber. This was visible through the glass-panelled door and was used to monitor chamber temperature and relative humidity.

A Magnehelic pressure gauge (0-25 mm water gauge) was connected with each chamber by a nylon tube. This was mounted on the secondary dilution vessel trolley and was used to monitor the atmospheric pressure inside the chamber, relative to the exposure room. The internal pressure within each chamber was maintained in the range -2 to –4 mm water below ambient pressure when operational.

Extract flow was adjusted using gate valves mounted in the extract ducting between the chamber and filters.

Extraction of the chambers was accomplished by means of a single fan mounted on the outside wall of the building withdrawing air through a manifold to which all chambers were connected. The chamber air extract was vented to atmosphere *via* an exhaust stack.

PROCEDURE

A separate exposure chamber was used for each group. The Control animals were exposed using an identical exposure chamber to that used for the test groups.

Prior to the start of each exposure, the mass of test substance in each of the pressure vessels was checked to ensure there was sufficient material for the scheduled duration of generation.

The rats were transferred from the holding cages and placed into the individual compartments of the exposure cages. The animals were located on either level 2 or 3 of the 4 level chamber at one time. In order to avoid any variations in the dose received due to the spatial arrangements of the animals within the chamber, the position of the animals within the chamber was changed daily according to a previously assigned sequence.

The diluent and generator airflows were turned on and the exposure chamber doors were checked to ensure they were secured. The chamber pressure, relative to the exposure room was checked using each of the associated Magnehelic gauges to ensure that operation of the chamber took place at a slightly negative pressure.

The test substance supply toggle valves between the pressure vessel and the metering valves were opened; the exposure start time was noted and simultaneously the chamber environmental monitoring system was activated (see below). At intervals of 30 minutes, any clinical signs in the rats were recorded together with checks of generation and chamber operational parameters.

The wet and dry bulb temperatures of a thermohygrometer placed in each chamber were also recorded at approximately 30-minute intervals throughout each exposure. Relative humidity was found using a look-up table. The volume flow of air to the exposure chambers was measured using calibrated flow meters and also checked approximately every 30 minutes.

Results of the determination of Pyrolysis C5 in the chamber atmosphere were automatically recorded for each chamber at approximately half hourly intervals throughout each exposure.

At the end of six hours generation, the isolating toggle valves in the test substance supply lines were turned off and the weight of each pressure vessel and its residual contents was recorded. The vapour in the test chambers was allowed to clear for at least 15 minutes before the animals were removed.

At the end of this time, the rats were unloaded from the chambers and returned to their respective holding cages.

The chambers were washed with hot water.

A summary of the operating conditions is presented in Table A.

TEST ATMOSPHERE ANALYSIS

A gas chromatograph was used to measure the concentrations of Pyrolysis C5 in the test atmospheres within the four-inhalation chambers. Operating details of the Gas Chromatography system, its standardisation and validation are given in Appendix A.

The Gas Chromatograph was located adjacent to the exposure chambers.

The instrument was connected to each selected sampling port by programmed switching of valves under the control of the CEMS-2 program. Gas sampling lines were 0.6 cm diameter stainless steel tubing. A further set of automated valves admitted standards from gas sampling bags for calibration of the Gas Chromatograph and for daily checking of the standard response. To minimise the opportunity for carry over of the test substance within the sample lines, the conduit in which the sample stream passed to the Gas Chromatograph was purged with clean air for 60 seconds and further flushed with sample between analyses.

Before the start of each exposure, the operating conditions for the Gas Chromatograph were identified and the instrument response checked using prepared standard gas mixtures. An automatic warning message was generated in response to any deviation from the accepted response range for the standards.

Linear regression analysis of the Gas Chromatograph response to standards was incorporated into the system program to enable concentrations to be calculated from the signals provided by the chromatograph. The accumulated calibration data were reviewed at intervals during the study and, if necessary, the regression data incorporated into the program were revised. Details of such reviews are retained with the raw data.

CHAMBER MONITORING SYSTEM

A personal computer (PC) running the Chamber Environmental Monitoring System (CEMS-2) software was used to monitor and record the system performance during each exposure. The data collection sequence and display were controlled by a PC and all information collected was displayed on a monitor. Simultaneously, the data was stored electronically. This program was composed of three basic stages of operation: an initial setting up (pre-exposure) phase, an exposure monitoring phase and the post exposure data collation and presentation phase. The program is driven by a study data-protocol containing study specific design and detail. The CEMS-2 system holds a certificate of validation in compliance with GLP. All information collected was printed as a hard copy.

Setting-up phase

In the initial phase, prompted by the program screen display, study identification, dates, times and other relevant study details were entered and stored together with barometric pressure and gas chromatograph calibration data sets.

A pre-monitor calibration check using valid gasbag standards was conducted prior to the start of the exposure.

Exposure monitoring phase

This phase was started coincident with the commencement of generation. The concentration of Pyrolysis C5 in the chamber atmosphere of each test Group was analysed and recorded during a 30-minute cycle. The data were displayed on screen, printed and all downloaded to the database. This cycle of monitoring took place in the following sequence: High dose, Intermediate dose, Low dose and Air control and was repeated throughout the six-hour exposure period. A total of twelve cycles were recorded.

Post exposure phase

At the end of 6 hours, the data collected during exposure were collated into separate groups. The mean values, together with standard deviation were calculated for each parameter recorded. This data were printed and stored to the database. The first set of data was excluded from calculation of the mean because chamber concentrations did not stabilise until approximately 15 - 20 minutes from the start of exposure (equilibration time, t_{99} was 23 minutes).

TARGET CONCENTRATIONS

The target concentrations of Pyrolysis C5 were:

Group	Designation	Concentration (ppm)
2	Low dose	1000
3	Inter. dose	3000
4	High dose	5000

The target concentrations were selected in consultation with the Sponsor, following the review of available data.

EXPOSURE CHAMBER CONDITIONS

Chamber analysed concentration of Pyrolysis C5

Chamber atmosphere was sampled in sequence from each of the four exposure chambers (Chambers 4 - 1 sampled sequentially) and from one point within each chamber. Air from each chamber was continually drawn through a transfer line, which was therefore equilibrated with the mean concentration from each chamber. When not being sampled, these transfer lines were pumped to waste (Figure B).

Every seven minutes, air from the transfer lines was switched to the injection loop of the gas chromatograph for automated analysis and data processing.

The analytical methodology is presented in Appendix A.

Chamber spatial distribution

The spatial distribution of vapour in the chamber was checked during preliminary trials for this study. A difference in concentration of less than 1.5% with varying sample point was observed, which was well within the 10% tolerance limits normally allowed for this type of system. The manual sample points are shown in Figure B.

Nominal concentration of chamber atmospheres

Each chamber nominal concentration was calculated from the mass of liquid used over the six-hour exposure period and the exposure mean airflow. The ideal gas equation was used with the molecular weight of the liquid and the measured chamber conditions to compute the volume of vapour produced from the mass of liquid used. The calculation is detailed in Table C.

Airflow, temperature and relative humidity

These parameters were recorded manually, as described above under the Test Atmosphere generation and Procedure sections.

RESULTS

VAPOUR CONCENTRATION

Analysed concentration

The data are presented as follows:

Daily mean values	Table B
Individual values	Appendix B

The study mean concentration (the mean of daily mean values) for each group exposed to Pyrolysis C5 are presented below:

Group	Chamber concentration (ppm)	
	Target	**Analysed** [d]
2 (Low dose)	1000	983
3 (Inter. dose)	3000	2970
4 (High dose)	5000	5080

[d] Calculation standardised using Isoprene as the major component

Analysed concentrations were in very good agreement with the target concentrations. The coefficients of variation of the daily means were 6.6, 4.3 and 8.1% for Groups 2, 3 and 4 (Low, Intermediate and High doses) respectively.

A complete peak profile and data regarding the concentration both pentane and trans-1,3-pentadiene with respect to exposure duration and number are retained within the raw data.

Nominal concentration

The data are presented in Table C and are summarised below:

Group	Nominal concentration (ppm)	A/N ratio (%)
2 (Low dose)	1029	96.0
3 (Inter. dose)	3160	94.6
4 (High dose)	5927	95.9

$$A/N = \left(\frac{\text{Analysed concentration}}{\text{Nominal concentration}} \right) \times 100$$

For each Group, the nominal concentration for each exposure was calculated from the following parameters:

The mass of Pyrolysis C5 delivered into each vapour generator;
The mean chamber temperature;
The barometric (atmospheric) pressure;
The molecular weight of Pyrolysis C5;
The gas constant;
The chamber airflow;
The exposure duration.

The equations used for the calculation of the nominal concentration are detailed in Table C.

The mean ratios of analysed to nominal concentration (A/N), expressed as a percentage for the study, were between 94 and 96% for all dose groups with coefficients of variation less than 9%. This indicates consistency with vapourisation of the test material and little likelihood of residue being retained anywhere throughout the whole of the generation system. The A/N ratios for all dose groups were well within the acceptable tolerances for the dynamic vapour generation system used in this study. Differences from the ideal A/N of 100% maybe due to inaccuracies in the measurements of weight, airflow, temperature and analysed concentrations.

CHAMBER TEMPERATURE AND RELATIVE HUMIDITY

The daily mean chamber temperatures and relative humidities are presented in Table D.

The chamber temperatures were similar for all groups for most days of the study.

For Groups 1, 2 and 3 (Air Control, Low and Intermediate doses), the recorded RH was lower than the target range of 40 - 60%. The Group 4 (High dose) relative humidity mean was 41%, however, data was only collected for two exposures.

The low values of RH probably arise from generation and dilution of the chamber atmospheres with air that was supplied from a compressor system incorporating a refrigerant drier. This deviation from the target conditions had no discernible effect upon the animals and is not considered to have affected the outcome of the study.

DISCUSSION

Control of the delivery of Pyrolysis C5 vapour to the exposure chambers was excellent, as reflected in the study mean concentrations, which were within 2% of the target values for all groups.

The coefficients of variation for the daily mean concentrations were 6.6, 4.3 and 8.1% for Groups 2, 3 and 4 (Low, Intermediate and High doses) respectively. After initial problems with generation during the first exposure of Group 2 (Low dose), despite preliminary experimentation, the target concentration was repeatable for the duration of the study. The high coefficient of variation for Group 4 was the result of abandoning the exposures after the second day.

All individual samples in all exposures, collected from the Group 1 (Air control) chamber showed traces of Pyrolysis C5. This was considered to be "carryover" from the preceding sample in the common sampling line rather than the presence of Pyrolysis C5 within the exposure chamber. The mean amount present in terms of the peak area response was (on average) equivalent of 4 to 5% of the area response of the preceding Group 2 (Low dose) sample (target concentration 1000 ppm).

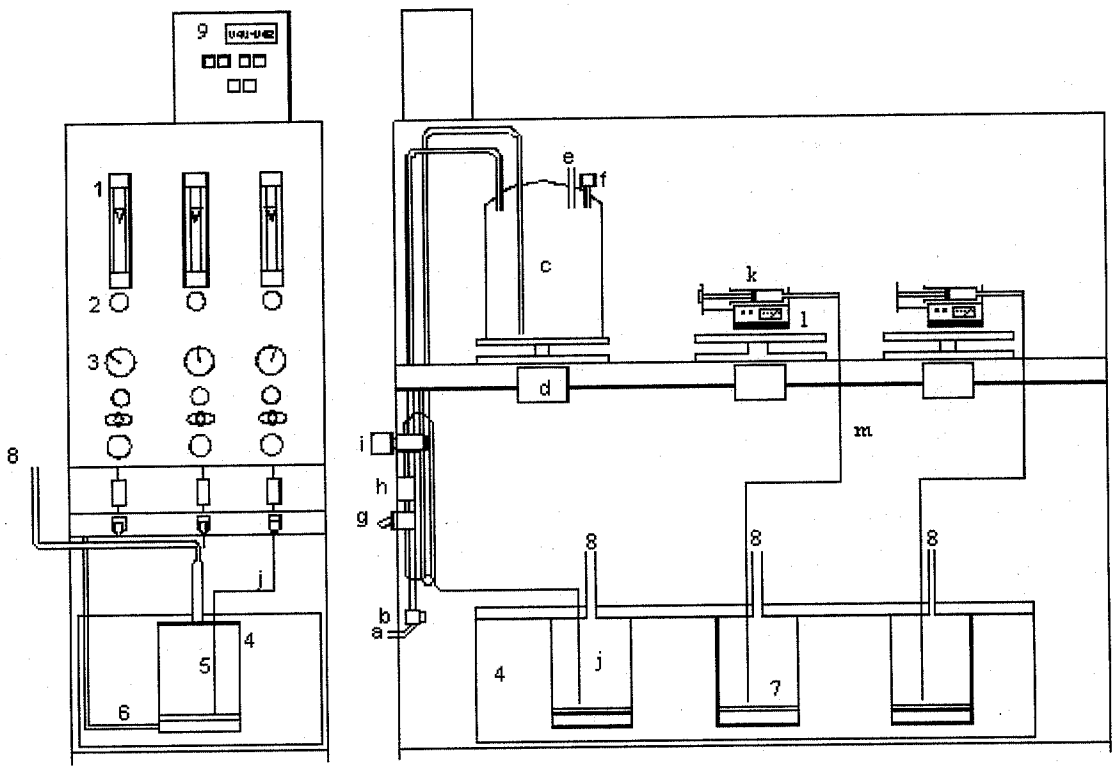
Very good agreement was also observed between the analysed and the nominal chamber concentration values for Groups 2, 3 and 4 (Low, Intermediate and High doses) respectively. Study mean analysed/nominal (A/N) ratios of 94 and 96% were observed.

CALCULATIONS

In order to minimise the cumulative errors which result from repeated rounding of numbers, much of the data in this report has been calculated continuously using unrounded numbers and only rounded for printing. Consequently, any further calculations using these rounded numbers may include rounding errors in the last significant figure, possibly leading to small apparent discrepancies with other data in the report.

FIGURE A

Schematic of a vapour generation system

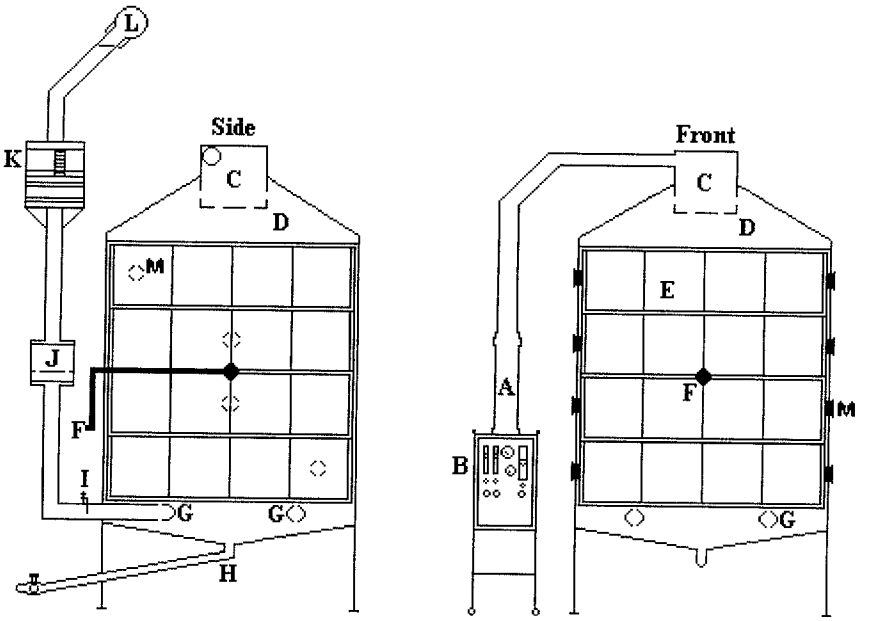


Key

1	Diluent air rotameters	a	Test substance inlet from bulk source
2	Rotameter control valve	b	Test substance feed line filters
3	Reservoir pressure display	c	Liquid reservoir
4	Water bath	d	Load cell
5	Vapour generator	e	Helium (pressure) inlet
6	Diluent air	f	Safety valve
7	Sinter Diameter (130 mm)	g	Toggle valve
8	Vapour/air mixture (to chamber)	h	0.5 μm stainless steel filter
9	Load cell display	i	Micro-metering needle valve
		j	Liquid line to vapour generator
		k	Polypropylene syringe
		l	Syringe Driver (Precidor 5003)
		m	Test compound feed line

FIGURE B

Schematic of an inhalation chamber used to expose rats



Key

A	Vapour inlet pipe	G	Exhaust plenum
B	Air flow control and chamber monitoring	H	Drain
		I	Gate valve
C	Dispersion device	J	Pre-filter
D	Exposure chamber (0.75 m^3)	K	Powered extract filter
E	Animal exposure cages	L	Main exhaust
F	CEMS sampling port	M	Manual Sampling ports

TABLE A

Operating conditions for the rodent inhalation exposure system

Parameter	Group			
	1 (Air control)	2 (Low dose)	3 (Inter. dose)	4 (High dose)
Target concentration of Pyrolysis C5 (ppm)	0	1000	3000	5000
Chamber airflows (l/minute)				
Target air flow to vapour generator	N/A	50	90	150
Target supplementary airflow	150	100	60	N/A
Vapour generator settings				
Reservoir pressure (Helium, psi)	N/A	5	10	10
Sinter diameter (mm)	130	130	130	130
Chamber negative pressure (mm/H_2O)	4	4	4	4

N/A Not applicable

TABLE B

Chamber concentrations of Pyrolysis C5 (ppm) - daily mean values

Exposure No.	Chamber Concentration (ppm)			
	Gp1 (Control	Gp2 (Low Dose)	Gp3 (Inter. Dose)	Gp4 (high Dose)
1	BLQ	850	2853	4790
2	BLQ	1061	3167	5369
3	BLQ	987	2883	
4	BLQ	940	2927	
5	BLQ	1023	3018	
6	BLQ	976		
7	BLQ	1002		
8	BLQ	1026		
Mean of Means	BLQ	983	2970	5080
sd		64.8	126.7	409.2
CV		6.6	4.3	8.1

BLQ The peak detected was below the limit of quantification (LOQ) for the assay. The LOQ was set at 560 ppm (20% below the lowest calibration standard (nominally 700 ppm)).

sd Standard deviation

CV Coefficient of variation (sd × 100/mean)

TABLE C

Nominal concentrations of Pyrolysis C5 (ppm) - individual exposure values

Group 2 (Low dose) - Target concentration 1000 ppm

Exposure		Barometric pressure (mmHg)	Pyrolysis C5 usage (kg)	Chamber Airflow [e] (l/minute)	Chamber concentration		A/N ratio (%)
No.	Duration (minute)				Nominal [f] (ppm)	Analysed (ppm)	
1	360	765	0.163	150	850	1035	82.1
2	360	760	0.189	150	1061	1207	87.9
3	360	753	0.140	150	987	904	109.2
4	360	760	0.146	150	940	933	100.7
5	360	768	0.158	150	1023	1001	102.2
6	360	757	0.158	150	976	1012	96.5
7	360	758	0.163	150	1002	1046	95.8
8	360	756	0.170	150	1026	1093	93.9
Mean of Means		760	0.161	150	983	1029	96.0
sd		4.9	0.0149	0.0	64.9	94.2	8.43
CV		0.6	9.3	0.0	6.6	9.2	8.8

[e] Includes 60 l/minute through vaporiser

[f] Calculated from the following equations:

$$\text{Concentration (ppm)} = \frac{V}{V_a + V} \times 10^6$$

$$V = \frac{W \times R \times T}{M} \times \frac{760\ \text{mm Hg}}{\text{Atm}}$$

where V = gaseous volume of Pyrolysis C5 (litres)
W = mass of Pyrolysis C5 (kg)
M = molecular weight of Pyrolysis C5 (assumed 70 g/mole)
R = gas constant (0.08205 l atm mol^{-1} K^{-1})
T = temperature (K), = temperature (°C, see Table D) + 273
Atm = atmospheric pressure (mmHg)
V_a = volume of air (litres) passing through the chamber during the exposure

A/N Analysed/nominal concentration ratio expressed as a percentage
sd Standard deviation
CV Coefficient of variation (sd × 100/mean)

TABLE C

(Nominal concentrations of Pyrolysis C5 (ppm) - individual exposure values - continued)

Group 3 (Intermediate dose) - Target concentration 3000 ppm

Exposure		Barometric pressure (mmHg)	Pyrolysis C5 usage (kg)	Chamber Airflow [g] (l/minute)	Chamber concentration		A/N ratio (%)
No.	Duration (minute)				Nominal [f] (ppm)	Analysed (ppm)	
1	360	765	0.54	150	2853	3423	83.3
2	360	760	0.56	150	3167	3574	88.6
3	360	753	0.46	150	2883	2964	97.3
4	360	760	0.46	150	2927	2938	99.6
5	360	768	0.46	150	3018	2903	104.0
Mean of Means		761	0.50	150	2970	3160	94.6
sd		5.7	0.050	0.0	126.7	314.1	8.40
CV		0.8	10.0	0.0	4.3	9.9	8.9

[f] Calculated from the following equations:

$$\text{Concentration (ppm)} = \frac{V}{V_a + V} \times 10^6$$

$$V = \frac{W \times R \times T}{M} \times \frac{760\ \text{mm Hg}}{\text{Atm}}$$

where V = gaseous volume of Pyrolysis C5 (litres)
W = mass of Pyrolysis C5 (kg)
M = molecular weight of Pyrolysis C5 (assumed 70 g/mole)
R = gas constant (0.08205 l atm mol^{-1} K^{-1})
T = temperature (K), = temperature (°C, see Table D) + 273
Atm = atmospheric pressure (mmHg)
V_a = volume of air (litres) passing through the chamber during the exposure

[g] Includes 90 l/minute through vaporiser
A/N Analysed/nominal concentration ratio expressed as a percentage
sd Standard deviation
CV Coefficient of variation (sd × 100/mean)

TABLE C

(Nominal concentrations of Pyrolysis C5 (ppm) - individual exposure values - continued)

Group 4 (High dose) - Target concentration 5000 ppm

Exposure		Barometric pressure (mmHg)	Pyrolysis C5 usage (kg)	Chamber Airflow [h] (l/minute)	Chamber concentration		A/N ratio (%)
No.	Duration (minute)				Nominal [f] (ppm)	Analysed (ppm)	
1	360	765	0.84	150	4790	5306	90.3
2	360	760	0.83	150	5369	5288	101.5
Mean of Means		763	0.84	150	5080	5297	95.9
sd		3.5	0.007	0.0	409.4	12.6	7.96
CV		0.5	0.8	0.0	8.1	0.2	8.3

[f] Calculated from the following equations:

$$\text{Concentration (ppm)} = \frac{V}{V_a + V} \times 10^6$$

$$V = \frac{W \times R \times T}{M} \times \frac{760\,\text{mm Hg}}{Atm}$$

where V = gaseous volume of Pyrolysis C5 (litres)
W = mass of Pyrolysis C5 (kg)
M = molecular weight of Pyrolysis C5 (assumed 70 g/mole)
R = gas constant (0.08205 l atm mol^{-1} K^{-1})
T = temperature (K), = temperature (°C, see Table D) + 273
Atm = atmospheric pressure (mmHg)
V_a = volume of air (litres) passing through the chamber during the exposure

[h] Includes 150 l/minute through vaporiser
A/N Analysed/nominal concentration ratio expressed as a percentage
sd Standard deviation
CV Coefficient of variation (sd × 100/mean)

TABLE D

Chamber temperature and relative humidity - exposure mean values

Exposure No.	Mean chamber temperatures (°C) and relative humidity (%RH)							
	Group 1 (Air control)		Group 2 (Low dose)		Group 3 (Intermediate dose)		Group 4 (High dose)	
	Temp	RH	Temp	RH	Temp	RH	Temp	RH
1	21.6	35	21.6	26	21.8	33	21.3	36
2	21.6	35	21.5	29	21.9	44	21.9	45
3	21.8	31	21.9	26	21.8	36		
4	21.9	31	21.5	30	21.9	42		
5	21.9	30	22.0	27	21.5	41		
6	21.0	33	21.0	28				
7	21.5	33	21.9	27				
8	21.9	34	21.8	33				
Mean	21.7	32	21.7	28	21.8	39	21.6	41
sd	0.31	2.1	0.33	2.4	0.16	4.5	0.42	6.4
CV (%)	1.4	6.5	1.5	8.5	0.8	11.6	2.0	15.7

sd standard deviation

CV Coefficient of variation (sd × 100/mean)

APPENDIX A

Methods of sample collection and analysis for Pyrolysis C5

SAMPLE COLLECTION

Chamber concentration

Samples of chamber air were collected in sequence from each of the four exposure chambers (Chambers 4 - 1 sampled sequentially) from one sampling point within each chamber. Air from each chamber was continually drawn through a transfer line, which was therefore equilibrated with the mean concentration from each chamber. When not being sampled, the air from the transfer lines was pumped to waste.

At approximately seven-minute intervals, the air from the transfer lines was switched to the injection loop of the Gas Chromatograph for automated analysis and data processing.

METHOD OF ANALYSIS

Chamber atmosphere samples were analysed by gas chromatography. The method of sample analysis is detailed, together with a summary of the method validation, in the Inhalation Analytical Procedure at the end of this Appendix.

APPENDIX A

(Methods of sample collection and analysis for Pyrolysis C5 - continued)

CALCULATIONS

GC analysis

The samples of chamber atmosphere were injected into a gas chromatograph, which was calibrated using vapour standards prepared in gas sampling bags. The method for calculating the concentration of Pyrolysis C5 from the mass used to prepare each vapour standard is given below in equations 1 and 2.

$$\text{Concentration} = \frac{V}{V_a + V} \times 1{,}000{,}000 \text{ ppm} \qquad (1)$$

$$V = \frac{W \times R \times T}{M} \times \frac{760}{Atm} \qquad (2)$$

where V = gaseous volume of Pyrolysis C5 (ml)
W = mass of Pyrolysis C5 (mg)
M = molecular weight of Pyrolysis C5 (assumed 70 g/mole)
R = Gas constant (0.08205 ml atm $mmol^{-1}$ K^{-1})
T = temperature (K)
Atm = atmospheric pressure (mmHg)
V_a = volume of air (ml)

APPENDIX A

(Methods of sample collection and analysis for Pyrolysis C5 - continued)

COMPOUND SPECIFIC INHALATION ANALYTICAL PROCEDURE FOR ISOPRENE IN PYROLYSIS C5 VOLATILES

The analysis of isoprene in Pyrolysis C5 volatiles in air

The method outlined in this document has been validated and is considered fit for the purpose of monitoring test atmospheres and blood headspace in an Inhalation Toxicology study.

This document details the basic procedures for the analysis of isoprene in Pyrolysis C5 sampled by an automated on-line sampling system directly from the inhalation chambers. The resulting samples, of approximate concentration 700 to 7000 ppm, are analysed by GC. Study specific amendments and additions will be detailed within a supplementary document.

EFFECTIVE DATE:	3 March 2002

Test substance

Pyrolysis C5 is a mixture of mostly C5 hydrocarbons with some higher and lower homologues and has an approximate molecular weight of 70. The major component is isoprene (2-methyl-1,3-butadiene) and standardisation is calculated using the area response for this peak. The two other major components are n- pentane and *trans*-1,3-pentadiene. The composition of Pyrolysis C5 is analysed through the course of the exposure by measurement of the peak area of these components in comparison to that of isoprene.

Appearance	Colourless liquid
Storage	A pressure vessel in a cold storage facility at 10°C

APPENDIX A

(Methods of sample collection and analysis for Pyrolysis C5 - continued)

Equipment

Balance and data printer	Sartorius	R200D with YDP-02
Syringes	Hamilton	500 series gas-tight (500 ml)
Gas sample bags	SKC INC	Tedlar® 232-series (1 and 3 dm^3 capacity)
Syringe valve	Mininert	Push button valve
Vacuum pump	AEG	ADEB 56 (or equivalent)
Flow meter	J & W Scientific	ADM1000 (acoustic displacement)
Wet gas meter	Zeal	DM3B (1 l)

APPENDIX A

(Methods of sample collection and analysis for Pyrolysis C5 - continued)

Preparation of samples for analysis

Gas samples

Vapour samples are collected using an automated system fitted with electronically controlled valves, which are manipulated using the Chamber Environment Monitoring System (CEMS-2) software.

The test atmosphere is drawn directly from the inhalation chamber through the sample line to the gas-sampling valve located on the GC.

Initially, the gas-sampling valve of the GC is set to the "load" position and the valve is automatically switched to the "inject" position after 60 seconds. Simultaneously, the GC activates the start of the run sequence.

Preparation of calibration standards

Gas standards

Standards are prepared using the following method, the actual standard concentration ranges used are as detailed in the study specific supplement.

Collect the liquid sample directly from the storage cylinder into an empty glass bottle. Some of this liquid sample is then transferred to a glass vial fitted with a sealing valve. The glass vial is then stored at 4°C.

Evacuate gas sample bags of appropriate volumes and introduce measured volumes of air using a wet gas meter. Use a gas tight syringe fitted with a sealing valve to accurately dispense an aliquot of Pyrolysis C5 into the top standard gas bag via the injection port. Using a gas tight syringe, accurately dispense measured volumes of the Pyrolysis C5 vapour from the top standard gas bag into the gas sample bags via the injection port to produce standards covering the concentration range described in the study specific supplement.

APPENDIX A

(Methods of sample collection and analysis for Pyrolysis C5 - continued)

Storage of standards and samples

The maximum storage periods for the various sample types are detailed below:

Sample type	Storage conditions	Storage period
Gas standards	Room temp., dark	3500-700ppm: 8 days 7000ppm: 7 days

Calibration and quantification

Gas analysis

Calibrate by injecting duplicates of each calibration standard gas bag, as detailed in the study specific supplement, at the beginning of each analytical sequence. Measure the peak area response of the major component peak (isoprene) in each injection of the calibration standard gas bag and derive the line of best fit using a 1/concentration^2 weighted least squares method.

For each injection of the sample measure the peak area response and determine the amount present in the sample using the equation below:

$$\text{Amount(ppm)} = \frac{(A - I)}{S}$$

Where A = Peak area response of isoprene in Pyrolysis C5 in the sample chromatogram
S = Slope of calibration line derived from calibration data
I = Intercept of calibration line derived from calibration data

At the beginning of each analytical sequence, conduct a "Pre-Monitor Calibration check" to ensure the gas bags are within accepted tolerance limits of the calibration model i.e. run four QC standards covering the nominal range (including the LOQ) prior to the start of sample analysis.

APPENDIX A

(Methods of sample collection and analysis for Pyrolysis C5 - continued)

Chromatographic conditions

Analytical column	PE-5 (100% dimethyl polysiloxane), 25 m x 0.25 mm i.d. 0.25 μm film
Carrier gas	Helium (1.0 ml/min)
Split vent	Helium (49 ml/min)
Septum purge	Helium (2.6 ml/min)
Split ratio	1:50
Make up	Helium (30 ml/min)
Oxidant	Air (470 ml/min)
Fuel	Hydrogen (50 ml/min)
Injection volume	250 μl *via* an automated gas valve
Injector temperature	100°C
Detector temperature	250°C
Column temperature	Isothermal at 0°C for 5 minutes. Temperature controlled by liquid CO_2 delivered using a cryogenic GC unit
Retention time	n-pentane peak of Pyrolysis C5 mixture aprroxomately 2.95 minutes; Isoprene peak approximately 3.15 minutes, trans-1,3-pentadiene approximately 3.50 minutes

APPENDIX A

(Methods of sample collection and analysis for Pyrolysis C5 - continued)

Quality assurance measures

Gas analysis

When the method is established on a chromatographic system six injections of a standard will be used to verify performance of the system. The parameters and acceptance criteria are set out below:

Parameter	Acceptance criteria
Repeatability (CV, n=6)	<5%
Repeatability at LOQ (CV, n=6)	<10%
QC tolerance	< ±5%
QC tolerance at LOQ	< ±10%

The highest calibration standard will be compared against a standard of similar concentration prepared independently. The ratio of response factors will be acceptable if within the range 0.95 to 1.05.

A Pre-monitor calibration check, must precede every exposure for the analysis to be regarded as valid. The results of the quality check standards must lie within the QC tolerance limits.

A quality check standard of low concentration will be run to verify the LOQ for the run. The LOQ for the run will be regarded as the concentration of the lowest acceptable quality check standard.

APPENDIX A

(Methods of sample collection and analysis for Pyrolysis C5 - continued)

Summary of method validation

The raw data for the method validation is located in study CSS/011.

Comparison of test blanks, standards and test samples showed that the isoprene peak was adequately resolved from any potential interfering peak.

Precision data showed coefficients of variation for isoprene in Pyrolysis C5 of less than 5% with solutions in the range of 7000 to 700 ppm.

Least squares regression analysis with a 1/concentration^2 weighting of the peak area response against concentration of standard (700 to 7000 ppm) produced a correlation coefficient of 0.99999 and relative errors less than 5% in the range 7000 to 700 ppm. The Limit of Quantification (LOQ) for isoprene in Pyrolysis C5 will be set by the lowest acceptable check standard, however, the LOQ and Limit of Detection (LOD) are potentially as low as 186 and 61 ppm respectively (calculated statistically using the standard deviation obtained for a solution of concentration 700 ppm).

Standards of isoprene in Pyrolysis C5 at 7000 ppm stored at room temperature for 7 days and subsequently analysed against fresh standards showed concentrations within 5% of their nominal concentrations. Standards of isoprene in Pyrolysis C5 in the range 700 to 3500 ppm stored at room temperature for 8 days and subsequently analysed against fresh standards showed concentrations within 5% of their nominal concentrations.

Intermediate precision data showed an overall coefficient of variation of 2.3% and a difference in the mean result of 4.1% for the analysis of two batches of 6 samples of isoprene in Pyrolysis C5.

APPENDIX A

(Methods of sample collection and analysis for Pyrolysis C5 - continued)

Chromatographs

System No.	Components of gas chromatography system		
	Manufacturer	**Model No.**	**Description**
1	Hewlett Packard	5890A	Chromatograph with capillary inlets, heated automatic gas sampling valve, ECD and FID.
	Hewlett Packard	G1513A	Autoinjector }
	Hewlett Packard	18596CX	Controller }6890 Series Autosampler
	Hewlett Packard	G1512AX	Turntable }
	Thermo Finnegan [1]	SP4500	A/D interface
	Thermo Finnegan	PC1000	Integration software
2	Pye Unicam	PU4550	Chromatograph with gas valve and FID.
	Pye Unicam	PU4700	Autosampler
	Thermo Finnegan	SP4500	A/D interface
	Thermo Finnegan	PC1000	Integration software
3	Shimadzu	GC-14A	Chromatograph with FID.
	Shimadzu	AOC-1400	Autosampler
	Shimadzu	AOC-14	Autoinjector
	Shimadzu		Split injection system
	Thermo Finnegan	SP4500	A/D interface
	Thermo Finnegan	PC1000	Integration software
6	Shimadzu	GC-14A	Chromatograph with FID.
	Shimadzu	MGS-4	Automated gas valve
	Shimadzu	SPL-14A	Split injection system
	Shimadzu	CR4-A	Integrator
7	Shimadzu	GC-14A	Chromatograph with FID.
	Shimadzu	MGS-4	Automated gas valve
	Shimadzu	SPL-14A	Split injection system
	Shimadzu	CR4-A	Integrator
8	Hewlett Packard	5890A	Chromatograph with capillary inlets, heated automated gas sampling valve and FID.
	Hewlett Packard	18593B	Autoinjector }
	Hewlett Packard	18596CX	Controller }7673 Autosampler
	Hewlett Packard	G1512AX	Turntable }
	Thermo Finnegan	SP4500	A/D interface
	Thermo Finnegan	PC1000	Integration software
9	Perkin Elmer	Autosystem XL	Automatic Chromatograph with programmable split/less capillary injector, heated automatic gas sampling valve and FID.

[1] Thermo Finnegan has previously traded as ThermoQuest, Thermo Separation Products (TSP) and Spectra Physics. Individual equipment items and manuals may be identified with these trade-names.

ECD Electron capture detector
FID Flame ionisation detector

APPENDIX A

(Methods of sample collection and analysis for Pyrolysis C5 - continued)

CSS/011 - STUDY SPECIFIC SUPPLEMENT TO THE INHALATION ANALYTICAL PROCEDURE FOR ISOPRENE IN PYROLYSIS C5

This supplement details additions and amendments to the procedure to be used for the GC assay of Isoprene in Pyrolysis C5 obtained from air samples collected on the above study.

The assay, incorporating the additions and amendments, is suitable for the analysis of Isoprene in Pyrolysis C5, in solution, at concentrations within the range of 700 to 7000 ppm.

Details given in this supplement supersede those in the compound specific IAP.

EFFECTIVE DATE :	**3 March 2002**

Analytical standard

Name	Isoprene pentadiene
Batch number	None supplied
Purity	See below
Expiry date	None supplied
Supplier	Dow Chemical

Composition of Pyrolysis C5

Component name	**Weight %**
Isopentane	8.01
1,4-pentadiene	2.64
2-butyne	0.95
1-pentene	6.34
2-methyl-1-butene	3.59
n-pentane	9.52
Isoprene	17.84
Trans-2-pentene	2.83
Cis-2-pentene	1.78
2-methyl-2-butene	2.51
Trans-1,3-pentadiene	10.00
Cyclopentadiene	7.70
Cis-1,3,-pentadiene	6.12
Cyclopentene	7.01
Cyclopentane	1.33
2-Methylpentane	1.73
Dicyclopentadiene	5.87

APPENDIX A

(Methods of sample collection and analysis for Pyrolysis C5 - continued)

Preparation of standard solutions

Prepare standard gas bags in the nominal range 700 to 7000 ppm.

Calibration and Quantification

Calibration of the instrument is performed using standard gas bags in the nominal range 700 to 7000 ppm.

Chromatographs

The analysis is performed using chromatograph 6.

APPENDIX B

Individual Pyrolysis C5 concentration measurements

Exposure No.	Sample No.	Chamber Concentration (ppm)			
		Group 1 (Air control)	Group 2 (Low dose)	Group 3 (Inter. dose)	Group 4 (High dose)
1	1	BLQ	BLQ[j]	2234	1590
	2	BLQ	350[j]	2393	4870
	3	BLQ	350[j]	2420	4705
	4	BLQ	350[j]	3099	4928
	5	BLQ	665[k]	2936	4859
	6	BLQ	1609[k]	2993[k]	4675[k]
	7	BLQ	675	3072	4844
	8	BLQ	1159	2902	4730
	9	BLQ	1189	2969	4785
	10	BLQ	1108	2869	4761
	11	BLQ	1049	2878	4746
	12	BLQ	[BLQ][l]	[BLQ][l]	[1768][l]
	Mean[m]	BLQ	850	2853	4790
	sd		435.6	247.5	81.4

BLQ The peak detected was below the limit of quantification (LOQ) for the assay. The LOQ was set at 560 ppm (20% below the lowest calibration standard (nominally 700 ppm)).

j Below the limit of quantification (BLQ) - The analytical value recorded was below the limit of quantification (LOQ) for the analytical sequence. Where a BLQ result is recorded for an individual sample, to avoid adversely affecting the data by entering a zero value, a value calculated using the following equation was used.

$$\text{BLQ value(ppm)} = \frac{\text{Concentration of standard at LOQ (ppm)}}{2}$$

The standard at LOQ is the concentration of the lowest acceptable QA standard used in the analytical sequence.

k Values extrapolated from calibration data after initial non detection.

l Values excluded from mean and standard deviation due to analysis problem.

m The initial concentration measurement of each exposure was excluded from all calculations.

sd Standard deviation

APPENDIX B

(Individual Pyrolysis C5 concentration measurements - continued)

Exposure No.	Sample No.	Chamber Concentration (ppm)			
		Group 1 (Air control)	Group 2 (Low dose)	Group 3 (Inter. dose)	Group 4 (High dose)
2	13	BLQ	993	2518	1287
	14	BLQ	1006 [k]	3155 [k]	5340 [k]
	15	BLQ	1027 [k]	3247 [k]	5332 [k]
	16	BLQ	1029	3115	5490
	17	BLQ	1106	3194	5336
	18	BLQ	1044	3252	5420
	19	BLQ	1078	3166	5343
	20	BLQ	1039	3035	5385
	21	BLQ	1030	3104	5312
	22	BLQ	1137	3125	5447
	23	BLQ	1095	3154	5124
	24	BLQ	1083	3290	5530
	Mean [m]	BLQ	1061	3167	5369
	sd		40.7	74.7	108.1

BLQ The peak detected was below the limit of quantification (LOQ) for the assay. The LOQ was set at 560 ppm (20% below the lowest calibration standard (nominally 700 ppm)).

[k] Values extrapolated from calibration data after initial non detection.

[m] The initial concentration measurement of each exposure was excluded from all calculations.

sd Standard deviation

APPENDIX B

(Individual Pyrolysis C5 concentration measurements - continued)

Exposure No.	Sample No.	Chamber Concentration (ppm)			
		Group 1 (Air control)	Group 2 (Low dose)	Group 3 (Inter. dose)	Group 4 (High dose)
3	25	BLQ	BLQ	2118	
	26	BLQ	638	2582	
	27	BLQ	1496	2484	
	28	BLQ	1050	2676	
	29	BLQ	1071	3496	
	30	BLQ	1081	2852	
	31	BLQ	1180	2880	
	32	BLQ	1475	2933	
	33	BLQ	350 [j]	2936	
	34	BLQ	715	2929	
	35	BLQ	897	2982	
	36	BLQ	904	2967	
	Mean [m]	BLQ	987	2883	
	sd		342.1	263.3	

BLQ The peak detected was below the limit of quantification (LOQ) for the assay. The LOQ was set at 560 ppm (20% below the lowest calibration standard (nominally 700 ppm)).

j Below the limit of quantification (BLQ) - The analytical value recorded was below the limit of quantification (LOQ) for the analytical sequence. Where a BLQ result is recorded for an individual sample, to avoid adversely affecting the data by entering a zero value, a value calculated using the following equation was used.

$$\text{BLQ value(ppm)} = \frac{\text{Concentration of standard at LOQ (ppm)}}{2}$$

The standard at LOQ is the concentration of the lowest acceptable QA standard used in the analytical sequence.

m The initial concentration measurement of each exposure was excluded from all calculations.

sd Standard deviation

APPENDIX B

(Individual Pyrolysis C5 concentration measurements - continued)

Exposure No.	Sample No.	Chamber Concentration (ppm)			
		Group 1 (Air control)	Group 2 (Low dose)	Group 3 (Inter. dose)	Group 4 (High dose)
4	37	BLQ	638	2116	
	38	BLQ	670	2605	
	39	BLQ	709	2678	
	40	BLQ	695	2653	
	41	BLQ	1052	2964	
	42	BLQ	1084	3040	
	43	BLQ	1106	3034	
	44	BLQ	1012	3060	
	45	BLQ	1002	3062	
	46	BLQ	1012	3047	
	47	BLQ	992	3009	
	48	BLQ	1010	3046	
	Mean [m]	BLQ	940	2927	
	sd		164.0	183.6	
5	49	BLQ	953	2474	
	50	BLQ	995	3012	
	51	BLQ	979	3003	
	52	BLQ	1033	3064	
	53	BLQ	1042	3081	
	54	BLQ	1059	3039	
	55	BLQ	1057	3103	
	56	BLQ	1012	2950	
	57	BLQ	1001	2964	
	58	BLQ	1041	2988	
	59	BLQ	1009	2974	
	60	BLQ	[BLQ] [l]	[BLQ] [l]	
	Mean [m]	BLQ	1023	3018	
	sd		27.3	52.2	

BLQ The peak detected was below the limit of quantification (LOQ) for the assay. The LOQ was set at 560 ppm (20% below the lowest calibration standard (nominally 700 ppm)).

[l] Values excluded from mean and standard deviation due to analysis problem.

[m] The initial concentration measurement of each exposure was excluded from all calculations.

sd Standard deviation

APPENDIX B

(Individual Pyrolysis C5 concentration measurements - continued)

Exposure No.	Sample No.	Chamber Concentration (ppm)			
		Group 1 (Air control)	Group 2 (Low dose)	Group 3 (Inter. dose)	Group 4 (High dose)
6	61	BLQ	902		
	62	BLQ	909		
	63	BLQ	952		
	64	BLQ	945		
	65	BLQ	954		
	66	BLQ	993		
	67	BLQ	990		
	68	BLQ	1008		
	69	BLQ	1006		
	70	BLQ	1001		
	71	BLQ	1006		
	72	BLQ	[BLQ] [l]		
	Mean [m]	BLQ	976		
	sd		34.0		
7	73	BLQ	916		
	74	BLQ	920		
	75	BLQ	968		
	76	BLQ	943		
	77	BLQ	951		
	78	BLQ	973		
	79	BLQ	979		
	80	BLQ	1054		
	81	BLQ	1039		
	82	BLQ	1059		
	83	BLQ	1093		
	84	BLQ	1045		
	Mean [m]	BLQ	1002		
	sd		57.2		

BLQ The peak detected was below the limit of quantification (LOQ) for the assay. The LOQ was set at 560 ppm (20% below the lowest calibration standard (nominally 700 ppm)).

l Values excluded from mean and standard deviation due to analysis problem.

m The initial concentration measurement of each exposure was excluded from all calculations.

sd Standard deviation

APPENDIX B

(Individual Pyrolysis C5 concentration measurements - continued)

Exposure No.	Sample No.	Chamber Concentration (ppm)			
		Group 1 (Air control)	Group 2 (Low dose)	Group 3 (Inter. dose)	Group 4 (High dose)
8	85	BLQ	908		
	86	BLQ	958		
	87	BLQ	977		
	88	BLQ	999		
	89	BLQ	1032		
	90	BLQ	1007		
	91	BLQ	1062		
	92	BLQ	1051		
	93	BLQ	1034		
	94	BLQ	1065		
	95	BLQ	1055		
	96	BLQ	1050		
	Mean [m]	BLQ	1026		
	sd		36.3		

BLQ The peak detected was below the limit of quantification (LOQ) for the assay. The LOQ was set at 560 ppm (20% below the lowest calibration standard (nominally 700 ppm)).

[m] The initial concentration measurement of each exposure was excluded from all calculations.

sd Standard deviation

STUDY PROTOCOL AND AMENDMENT

Study Number : **CSS/011**

CONFIDENTIAL

Huntingdon Life Sciences

PROTOCOL

PYROLYSIS C5

DOSE RANGE FINDING STUDY IN RATS

BY INHALATION EXPOSURE

Sponsor

American Chemistry Council
1300 Wilson Boulevard
Arlington
VA 22201
USA

Research Laboratory

Huntingdon Life Sciences Ltd
Woolley Road
Alconbury
Huntingdon
Cambridgeshire
PE28 4HS
ENGLAND

Total number of pages: 18

Final Protocol

Study Number : CSS/011

Huntingdon Life Sciences

CONTACT DETAILS

Sponsor's Representative	:	Elizabeth J. Moran, Ph.D., D.A.B.T.. Sponsors Representative for American Chemistry Council.

Study Number : **CSS/011**

Huntingdon Life Sciences

PROTOCOL APPROVAL

PYROLYSIS C5

DOSE RANGE FINDING STUDY IN RATS

BY INHALATION EXPOSURE

Amanda Brooker 19 February 2002

A.J. Brooker, B.Sc. (Hons.), M.Sc., C.Biol., M.I.Biol.
Study Director,
Huntingdon Life Sciences Ltd.

Date

The signature of the Study Director confirms this protocol as the working document for the study. Any changes made subsequent to the date of the Study Director's signature will be documented in formal amendments.

19 February 2002

D.W. Coombs, B.Sc., M.Sc.
Management,
Huntingdon Life Sciences Ltd.

Date

Feb 26 2002

Elizabeth J. Moran, Ph.D., D.A.B.T.
Sponsor,
American Chemistry Council

Date

Please sign both copies of this page, retain one for your records and return one to the Study Director at Huntingdon Life Sciences.

Study Number : **CSS/011**

Huntingdon Life Sciences

PYROLYSIS C5

DOSE RANGE FINDING STUDY IN RATS

BY INHALATION EXPOSURE

Enquiry Number: 21332L

Number of pages for internal distribution: 15

This working document is approved for circulation and use:

Amanda Brooker
A.J. Brooker B.Sc (Hons.), M.Sc., C.Biol., M.I.Biol.
Study Director

19 February 2002
Date

Primary location of study

Huntingdon Research Centre
Huntingdon
Cambridgeshire
PE28 4HS

Building Number: Y14, Room 011

All procedures to be performed at the above site.

Study Number : **CSS/011**

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Study Number : **CSS/011**

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1. INTRODUCTION

Management of study

Study Director	:	A.J. Brooker, B.Sc. (Hons.), M.Sc., C.Biol., M.I.Biol.
Consulting Toxicologist	:	C.J. Hardy, B.Sc., Ph.D., M.I.Biol., C.Biol., Dip.R.C.Path (Toxicology) (Inhalation aspects)
		Audrey Bottomley (Reproductive aspects)
In the temporary absence of the Study Director, the scientific responsibilities will be taken over by the Consulting Toxicologist; other items of routine study management should be referred to the following person in the first instance.	:	D.W. Coombs, B.Sc.

Objective

Assessment of influence on mated female rats and the outcome of pregnancy, to establish suitable dosages for a 4-week General Toxicity and Reproductive/Developmental Toxicity Screening study.

Good Laboratory Practice

The work performed in this study will generally follow good laboratory practice principles, however, no specific study-related Quality Assurance procedures will be performed and the report may not contain all of the elements required by GLP.

Animals (Scientific Procedures) Act 1986 compliance

The in-life experimental procedures to be undertaken during the course of this study are subject to the provisions of the United Kingdom Animals (Scientific Procedures) Act 1986 (the Act). The Act, administered by the UK Home Office, regulates all scientific procedures in living animals which may cause pain, suffering, distress or lasting harm and provides for the designation of establishments where procedures may be undertaken, the licensing of trained individuals who perform the practical techniques and the issue of project licences for specified programmes of work.

This study will comply with all applicable sections of the Act and the associated Codes of Practice for the Housing and Care of Animals used in Scientific Procedures and the Humane Killing of Animals under Schedule 1 to the Act, issued under section 21 of the Act.

The number of animals used will be the minimum that is consistent with scientific integrity and regulatory acceptability, consideration having been given to the welfare of individual animals in terms of the number and extent of procedures to be carried out on each animal.

Animal model	:	Female CD rat (sexually mature, virgin), requirement for a rodent species by regulatory agencies, extensively used at this laboratory.
Route	:	Inhalation, to simulate the conditions of human exposure.

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Treatment groups and dosages

Group	:	1	2	3	4
Compound	:	Control	---------- Pyrolysis C5 ----------		
Dosage (ppm)	:	0	*	*	*

* To be determined.

Dosage levels were based upon available data for the components.

2. STUDY SCHEDULE AND STRUCTURE

2.1. Duration of treatment

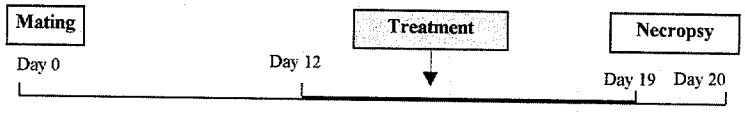
Females : Days 12 to 19 after mating.

2.2. Scheduled time plan

(to be up-dated as required in an amendment to protocol)

Sample of Pyrolysis C5 to arrive	:	January 2002	
Animals to arrive	:	15 February 2002	
Experimental start (Treatment to commence)	:	25 February 2002	
Experimental termination date (Animal sacrifice)	:	5 March 2002	
Draft report to be issued	:	April 2002	(estimated)

2.3. Study design



2.4. Identity of treatment groups

(to be selected from 26 animals ordered)

Group	Treatment	Dosage (ppm)#	Number of animals	Animal numbers
1	Control	0	6	1-6
2	Pyrolysis C5	*	6	7-12
3	Pyrolysis C5	*	6	13-18
4	Pyrolysis C5	*	6	19-24

* To be determined.
\# Expressed in terms of the test substance as supplied (ppm).

3. TEST SUBSTANCE AND ADMINISTRATION

In order for Huntingdon Life Sciences to comply with the Health and Safety at Work etc. Act 1974, and the Control of Substances Hazardous to Health Regulations 1999, it is a condition of undertaking the study that the Supplier shall provide Huntingdon Life Sciences with all information available to it regarding known or potential hazards associated with the handling and use of any substance supplied by the Supplier to Huntingdon Life Sciences. The Supplier shall also comply with all current legislation and regulations concerning shipment of substances by road, rail, sea or air.

Such information in the form of a completed Huntingdon Life Sciences test substance data sheet must be received by Safety Management Services at Huntingdon Life Sciences before the test substance can be handled in the laboratory. At the discretion of Safety Management Services at Huntingdon Life Sciences, other documentation containing the equivalent information may be acceptable.

Information received will be used to set the Huntingdon Life Sciences Hazard Class, which determines safety precautions taken in the workplace.

Huntingdon Life Sciences Hazard Class: [ ]

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3.1. Test substance

Sponsor's identification	:	Pyrolysis C5.
CAS Number	:	To be advised.
Storage conditions	:	At ambient temperature or in a refrigerator, unless otherwise directed by the Sponsor. Any such decision will be documented in the study data and included in the final report.
Supplier's responsibilities	:	Documentation of methods of synthesis, fabrication or derivation. Stability data. Certificate of analysis.
Certificate of analysis details	:	Test substance identity. Batch number. Purity. Composition. Other appropriate characteristics. Current expiry date.

A retention sample as required under 40 CFR 792.105(d) will not be taken nor held for the period specified by 40 CFR 792.195 as the test substance is not indefinitely stable and may also pose a safety hazard. The identity, other characterisitics and stability under conditions at the test site shall be documented by appropriate analyses prior to study(s)start and completion.

3.2. Atmosphere generation

Equipment	:	All glass vaporiser.
Method	:	The test substance (liquid) will be metered to the vaporiser (through which air is passed) from a reservoir pressurised with inert gas. The air supply is provided from a compressor and is filtered, temperature and humidity controlled. The vapour/air mixture produced passes directly into the exposure chamber. The exposure system will be fully detailed in the report.
Concentrations	:	To be determined.

3.3. Atmosphere sampling

Sampling : Samples of the test atmosphere from each exposure chamber will be drawn through a manifold by a diaphragm pump. At intervals, an automated valve system will divert a sample from each of the chambers in turn to a gas chromatograph. The samples will be injected onto a gas chromatograph (GC) by a motorised valve. The sampling frequency will be at least once per hour from each chamber of test atmosphere.

Concentration : The analysed concentration of significant components of the test atmospheres will be determined by capillary GC analysis with external standardisation. Spatial and temporal variations of concentrations in the chamber will be characterised during preliminary work without animals. Details of the analytical system will be confirmed by Protocol Amendment.

The nominal concentration will be determined from the amount of test material used and the airflow through the chamber.

3.4. Control of test atmosphere characteristics

Pre-study system characterisation : Before commencement of treatment the system will be characterised at the target exposure vapour concentrations without animals in order to:

- demonstrate reproducibility of vapour concentration.
- demonstrate homogeneity of vapour concentration and distribution between levels in the chamber.

4. ANIMAL MANAGEMENT

4.1. Animals - supply, acclimatisation and allocation

4.1.1. Animals

Species	:	Rat.
Strain	:	Crl:CD® BR.
Age range ordered	:	Females 9-10 weeks of age.
Weight range ordered	:	Females 200-220 g.
Supplier	:	Females: Charles River (UK) Limited. Margate, Kent, England.
Special	:	Timed-mated by supplier, day of detection of positive mating = Day 0 of pregnancy.

4.1.2. Acclimatisation

Duration	:	Approximately 10 days before treatment commences.
Husbandry conditions	:	Refer to Section 4.2.

4.1.3. Allocation to treatment groups

Allocation	:	On arrival.
Method	:	Random.
Possible exceptions	:	Allocation adjusted if arrival group mean gestation bodyweights differ beyond acceptable limits.
Cage distribution	:	Arrangement designed to minimise environmental variables.

4.1.4. Identification

Numbering	:	Unique for each animal within study.
Method	:	Tail tattoo.
Cage labels	:	Uniquely identifying the occupants.

4.1.5. Precommencement animal replacement

2 spare females will be ordered to replace any individuals rejected before the start of treatment.

Rejection before allocation	:	Ill health. Bodyweight extremes.
Replacement before treatment	:	All animals examined for general health and abnormalities on Day 11 of gestation. Any considered unsuitable may be replaced.
Replacement during treatment	:	None scheduled.

4.2. Animals - housing, diet and water supply

4.2.1. Environmental control

Rodent facility	:	Full barrier - to minimise entry of external biological and chemical agents.
Air supply	:	Filtered, not recirculated.
Temperature	:	Maintained within the range of 19-25°C.
Relative humidity	:	Maintained within the range of 40-70%.
Monitored continuously or daily.		Excursions outside these ranges documented in the study data.
Lighting	:	12 hours light : 12 hours dark.
Alarm systems	:	Activated on ventilation failure and when temperature/humidity limits exceeded.
Electricity supply	:	Public supply with automatic stand-by generators.

4.2.2. Animal accommodation

Non-exposure accommodation

Study period	Number of animals+		Cage material	Cage flooring
	Male	Female		
Gestation	-	Up to 3	Stainless steel	Stainless steel grid

+ Unless reduced by mortality or isolation.

Grid cages will be suspended above absorbent paper which will be changed at least twice each week. Cages, cage-trays, food hoppers and water bottles will be changed at appropriate intervals.

During exposure accommodation

The animals are transferred to separate individual cages in the exposure chamber during the 6 hour exposure period.

The individual animal exposure cages are of the suspended basket type, constructed of stainless steel mesh.

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4.2.3. Diet and water supply

Copies of all certificates of analysis are stored in the archives, and copies will be included in the final report.

Diet supply

Diet name	:	UAR VRF1 Certified.
Diet type	:	Pelleted diet.
Availability	:	Non-restricted.
Certification	:	Before delivery each batch of diet is analysed by the supplier for various nutritional components and chemical and microbiological contaminants. Supplier's analytical certificates are scrutinised and approved before any batch of diet is released for use.

Water supply

Supply	:	Public drinking water.
Regulatory agency	:	U.K. Department of the Environment.
Availability	:	Non-restricted via polyethylene or polycarbonate bottles with sipper tubes.
Certification	:	Certificates of analysis are routinely received from the supplier.

4.2.4. Contaminants assay

It is the Sponsor's responsibility to advise Huntingdon Life Sciences of any specific contaminants likely to prejudice the outcome of the study. Analyses for such contaminants may be performed if requested by the Sponsor.

4.3. Animals - procedures

4.3.1. Administration

Route	:	Inhalation by whole-body exposure in 0.75 m^3 exposure chambers.
Chamber conditions	:	Chamber temperature, humidity and air flow will be monitored and recorded at intervals during exposure.
Treated at	:	Constant air borne concentration.
Controls (Group 1)	:	Air.
Frequency	:	Once daily for 6 hours per day, for 8 consecutive days (Days 12 to 19 of gestation).

4.3.2. Clinical observations

Animals and their cages	:	Visually inspected at least twice daily for evidence of reaction to treatment or ill-health.
Deviations from normal recorded at the time in respect of	:	Nature and severity. Date and time of onset. Duration and progress of the observed condition.

In addition detailed observations will be made on days of exposure according to the following frequency:

1. Pre-exposure observation.
2. Observation during exposure (restricted to gross changes on a group basis).
3. Within ½ to 1 hour of return to home cage.

The above schedule will be amended, as necessary, in the light of signs observed.

During the acclimatisation period observations of the animals and their cages will be recorded at least once per day.

4.3.3. Mortality

Premature sacrifice	:	Animals may be killed on humane grounds or if considered *in extremis*.
Animals found dead, killed *in extremis* or on humane grounds	:	A necropsy is performed as soon as possible. Animals found outside the normal workday will be preserved in a refrigerator (approximately 4°C) provided for this purpose.

4.3.4. Bodyweight

Females	:	Days 2, 5, 8, 12, 16 and 20 after mating.

4.3.5. Food consumption

Recorded	:	Days 2-4, 5-7, 8-11, 12-15, 16-19 after mating.

5. NECROPSY AND FETAL PROCESSING

5.1. Time of necropsy

Day 20 after mating.

5.2. Method of kill

All adult animals	:	Inhaled carbon dioxide.
Fetuses	:	Chilling on a cool plate (approximately 0°C).

5.3. Macroscopic pathology

All adult females:

Macroscopic examination will be performed for evidence of disease or adverse reaction to treatment and abnormal tissues retained.

The number of corpora lutea in each ovary will be counted and the reproductive tract, complete with ovaries, will be dissected out. The following will be recorded:

Each ovary/uterine horn	:	Number of: Corpora lutea. Implantation sites. Resorption sites (early or late). Fetuses (live and dead). Fetuses and placentae dissected from the uterus and weighed individually. Fetuses sexed (external examination only).
Apparently non-pregnant animals	:	Status confirmed by Salewski staining technique for presence of implantation sites.
All fetuses	:	External examination only, then discarded.

5.4. Histology and light microscopy

(Optional)

Histological processing and microscopic examination of any retained tissues will only be performed, and documented in an amendment to the protocol, if requested by the Sponsor.

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6. DATA TREATMENT

6.1. Data processing

Where appropriate group mean values with standard deviation (SD) will be calculated from individual data:

Presentation of data

Bodyweight of adult females	:	Group mean values and SD calculated from individual data. Gain over relevant periods may be calculated and graphically presented.
Food consumption of adult females	:	Group mean values and SD calculated for the periods specified in Section 4.3.
Reproductive tract, Day 20 after mating	:	Corpora lutea. Implantations. Resorptions (early, late, total). Viable young (male, female, total). Sex ratio (male:female offspring).
Pre-implantation loss	:	$\frac{\text{Number corpora lutea} - \text{Number implantations}}{\text{Number corpora lutea}} \times 100$
Post-implantation loss	:	$\frac{\text{Number implantations} - \text{Number viable fetuses}}{\text{Number implantations}} \times 100$
Litter weight	:	From individual litter weights (tabulated).
Fetal weights	:	Group mean values and SD calculated for male, female and overall from - $\frac{\text{Total of individual litter mean fetal weights}}{\text{Number of litters}}$
Placental weights	:	Group mean values and SD calculated from - $\frac{\text{Total of individual litter placental weights}}{\text{Number of litters}}$

6.2. Statistical analysis

The small sample size precludes meaningful statistical evaluation. Inter-group differences will be assessed by reference to the control data.

7. REPORTING

Study progress : Periodic verbal and written updates on study progress will be provided by the Study Director.

Draft final report : For review by Sponsor.

Authorised final report : After approval from the Sponsor.

Routinely reports are supplied on US Quarto paper. The following numbers of reports are supplied:

Type of report	Printing	Number of copies		
		Bound	Unbound	Electronic
Draft report	Single-sided	0	2	1
Authorised final	Double-sided	1	0	1
	Single-sided	0	1	
Photographic report (if any)	Single-sided	1	0	

Any additions or corrections to an authorised final report will be documented as a formal addendum/amendment to the final report.

In the absence of ongoing communications, Huntingdon Life Sciences reserves the right to finalise, sign and issue the final report from this study six months after issue of the draft. In such an event, all materials will be transferred to the archive. Any subsequent requests for modifications, corrections or additions to the final report will be the subject of a formal report amendment (or new study, as appropriate) and will be subject to additional cost.

The data presented in the report will include but not be limited to the following:

Test substance information and analytical report

Strain, age, weight and source of animals used

Justification of administration route and rationale for dose level selection

Environmental conditions/animal husbandry and procedures

Certificates of analysis for diet and drinking water

Description of system and procedures for inhalation exposure

Results of the chamber concentrations

Clinical signs

Bodyweights

Food consumption

Litter measurements on Day 20 of gestation

Macroscopic pathology reports

Discussion/interpretation of results and conclusions

Study protocol and amendments

GLP compliance statement

8. QUALITY ASSURANCE AND ARCHIVING PROCEDURES

8.1. Quality assurance

No formal study-based Quality Assurance procedures will be performed on this study. These may be included if requested by the Sponsor – incorporated by protocol amendment.

8.2. Archiving

All raw data, samples and specimens arising from the performance of this study will remain the property of the Sponsor.

Types of sample and specimen which are unsuitable, by reason of instability, for long term retention and archiving may be disposed of after the periods stated in Huntingdon Life Sciences Standard Operating Procedures.

All other samples and specimens and all raw data will be retained by Huntingdon Life Sciences in its archive for a period of ten years from the date on which the Study Director signs the final report. After such time, the Sponsor will be contacted and his advice sought on the return, disposal or further retention of the materials. If requested, Huntingdon Life Sciences will continue to retain the materials subject to a reasonable fee being agreed with the Sponsor.

Huntingdon Life Sciences will retain the Quality Assurance records relevant to this study and a copy of the final report in its archive indefinitely.

Study Number : CSS/011
Protocol Amendment Number : 1

Huntingdon Life Sciences

PYROLYSIS C5

DOSE RANGE FINDING STUDY IN RATS

BY INHALATION EXPOSURE

Total number of pages: 3

Number of pages for internal distribution: 3

Study Director : A.J. Brooker, B.Sc. (Hons.), M.Sc., C.Biol., M.I.Biol.

The signature of the Study Director authorises the implementation of this amendment to protocol. In this amendment, deleted statements are struck through and new statements are underlined. Any changes to the study design after the date of this authorising signature will be documented in a further formal amendment.

AMENDMENT APPROVAL

For Huntingdon Life Sciences Ltd

Authorised by: Amanda Brooker Date: 27 February 2002
(Study Director)

For the Sponsor

Approved by: Date: March 7, 2002

Study Number : **CSS/011**
Protocol Amendment Number : **1**

Huntingdon Life Sciences

PYROLYSIS C5

DOSE RANGE FINDING STUDY IN RATS

BY INHALATION EXPOSURE

Reasons for amendments : Incorrect study room number detailed in protocol.
Inclusion of dosage levels.
Amended study dates due to supply of animals.
Addition of Huntingdon Life Sciences hazard class.

Amendments

Primary location of study

Building Number: Y14, Room ~~011~~ 010

1. INTRODUCTION

Treatment groups and dosages

Group	:	1	2	3	4
Compound	:	Control	---------- Pyrolysis C5 ----------		
Dosage (ppm)	:	0	1000 *	3000 *	5000 *

~~* To be determined.~~

Dosage levels were based upon available data for the components.

2.2. Scheduled time plan

(to be up-dated as required in an amendment to protocol)

Sample of Pyrolysis C5 to arrive	:	January 2002	
Animals to arrive	:	22 ~~15~~ February 2002	
Experimental start (Treatment to commence)	:	4 March ~~25 February~~ 2002	
Experimental termination date (Animal sacrifice)	:	12 ~~5~~ March 2002	
Draft report to be issued	:	April 2002	(estimated)

Huntingdon Life Sciences

2.4. Identity of treatment groups

(to be selected from 26 animals ordered)

Group	Treatment	Dosage (ppm)#	Number of animals	Animal numbers
1	Control	0	6	1-6
2	Pyrolysis C5	1000 *	6	7-12
3	Pyrolysis C5	3000 *	6	13-18
4	Pyrolysis C5	5000 *	6	19-24

~~* To be determined.~~

Expressed in terms of the test substance as supplied (ppm).

3. TEST SUBSTANCE AND ADMINISTRATION

Huntingdon Life Sciences Hazard Class: 2

3.2. Atmosphere generation

Concentrations : ~~To be determined.~~ 0, 1000, 3000 and 5000 ppm

CERTIFICATE OF ANALYSIS FOR RODENT DIET

CONTROL DATA

VRF1CP2.5 lot 10213

Date of Manufacture 2001/02/13

Sell by date 2001/06/13
Use by date 2002/02/13

Bag numbers ..... :	301 à 350

Quantity manufactured	*(tonnes)*	27
Variation from theoretical weight		*Conform*

SIEVE ANALYSIS (mm)

Diameter 0.00 - 0.10	0.2
Diameter 0.10 - 0.25	12.8
Diameter 0.25 - 0.50	44.3
Diameter 0.50 - 1.00	39.6
Diameter 1.00 - 2.00	3.1
Diameter 2.00 - 3.15	0.0
Diameter > 3.15	0.0

NUTRITIVE QUALITY

Incorporation of macro-mineral mix	*(Na)*	Positive	
Incorporation of micro-mineral premix	*(Mn and Cu)*	Positive	
Incorporation of vitamin premix	*(Vit.A and E)*	Positive	
Moisture	*(%)*	12.9	*(9 to 14)*
Crude protein	*(%)*	18.5	*(17.4 to 20.4)*
Crude oil	*(%)*	4.8	*(3.8 to 6.2)*
Nitrogen free extract	*(%)*	54.4	*(48.0 to 60.0)*
of which starch	*(%)*	40.4	
" which total sugars	*(%)*	4.1	
Crude fibre	*(%)*	3.6	*(2.8 to 5.2)*
Hemicellulose	*(%)*		
True cellulose	*(%)*		
Lignine	*(%)*		
Total minerals	*(%)*	5.8	*(4.5 to 7.0)*
Calcium	*(mg / Kg)*	8 700	*(8 000 to 12 000)*
Phosphorus	*(mg / Kg)*	7 200	*(4 000 to 8 300)*
Sodium	*(mg / Kg)*	2 800	*(2 500 to 3 700)*
Potassium	*(mg / Kg)*	8 100	*(5 700 to 9 700)*
Manganese	*(mg / Kg)*	64	*(20 to 100)*
Copper	*(mg / Kg)*	19	*(13 to 25)*
Vitamin A	*(UI / Kg)*	43 100	*(20 000 to 55 000*
Vitamin C	*(mg / Kg)*		
Vitamin E	*(mg / Kg)*	100	

CONTAMINENTS

BACTERIOLOGY			
Viable organisms	*(/ g)*	15 000	*(< 100 000)*
Moulds and yeasts	*(/ g)*	< 10	*(< 1 000)*
Total coliforms	*(/ g)*	0	*(< 5)*
Faecal coliforms	*(/ g)*	0	*(0)*
Anaerobies S.R.	*(/ g)*	< 10	*(< 100)*
Salmonella	*(/ 25 g)*	0	*(0)*

MYCOTOXINS *(µg / Kg)*		
Aflatoxin	< 1	*(< 5)*
Mycotoxin global risk	Negative	

Optional Notes

VRF1CP2.5 lot 10213

2001/02/13

HEAVY METALS

Lead - Pb .... (µg / Kg)	40	(< 1 500)
Mercury - Hg . (µg / Kg)	21	(< 100)
Arsenic - Ar . (µg / Kg)	10	(< 1 000)
Cadmium - Cd . (µg / Kg)	47	(< 250)
Selenium ..... (µg / Kg)	80	(< 600)

NITROGEN DERIVATIVES

NO2 ..... (mg / Kg)	< 0.5	
NO3 ..... (mg / Kg)	11.2	(Σ< 500)
NDMA ..... (µg / Kg)	1.9	(< 10)
NDEA ..... (µg / Kg)	< 0.2	(< 10)
NDPA ..... (µg / Kg)	< 0.3	(< 10)
NDBA ..... (µg / Kg)	< 0.3	(< 10)
NPIP ..... (µg / Kg)	< 0.3	(< 10)
NPYR ..... (µg / Kg)	< 0.5	(< 10)
NMOR ..... (µg / Kg)	< 0.6	(< 10)

PESTICIDES ORGANOS-CHLORINE (µg / Kg)

Lindane	< 1	(< 100)
a HCH	< 1	(< 20)
b HCH	< 5	(< 10)
d HCH	< 5	(< 100)
HCB	< 1	(< 10)
PCB	< 50	(< 50)
Aldrin	< 1	(< 10)
Dieldrin	< 1	(< 20)
Endosulfan	< 1	(< 100)

(Total < 200)

Heptachlor	< 1	
Heptachlor Epoxide	< 1	(Σ< 10)
Endrin	< 1	(< 10)
o.p'DDD	< 5	
p.p'DDD	< 5	
o.p'DDE	< 1	
p.p'DDE	< 1	(Σ< 50)
o.p'DDT	< 5	
p.p'DDT	< 5	

PESTICIDES ORGANOS-PHOSPHORUS (µg / Kg)

Acephate	< 500	(< 5 000)
Azinphos ethyl	< 50	(< 5 000)
Azinphos methyl	< 50	(< 5 000)
Bromophos ethyl	< 10	(< 5 000)
Bromophos methyl	< 20	(< 5 000)
Carbophenothion ethyl .	< 50	(< 5 000)
Carbophenothion methyl	< 20	(< 5 000)
Chlorfenvinphos	< 10	(< 5 000)
Chlormephos	< 10	(< 5 000)
Chlorpyriphos ethyl ...	< 15	(< 5 000)
Chlorpyriphos methyl ..	< 15	(< 1 500)
Chlorthiofos	< 15	(< 5 000)
Diazinon	< 15	(< 5 000)
Dichlofenthion	< 10	(< 5 000)
Dichlorvos	< 20	(< 5 000)
Diethion	< 10	(< 5 000)
Dimefox	< 20	(< 5 000)
Dimethoate	< 30	(< 1 000)
Dioxathion	< 15	(< 5 000)
Disulfoton	< 30	(< 5 000)
Ethoprophos	< 20	(< 5 000)
Fenchlorphos	< 20	(< 5 000)
Fenitrothion	< 15	(< 5 000)
Fenthion	< 30	(< 5 000)
Fonofos	< 20	(< 5 000)
Formothion	< 20	(< 5 000)
Heptenophos	< 30	(< 5 000)

(Total < 7 000)

Iodofenphos	< 25	(< 5 000)
Malathion	39	(< 5 000)
Methamidophos	< 15	(< 5 000)
Methidathion	< 25	(< 5 000)
Mevinphos	< 10	(< 5 000)
Monocrotophos	< 90	(< 5 000)
Naled	< 15	(< 5 000)
Oxydemeton methyl	< 400	(< 5 000)
Parathion ethyl	< 20	(< 5 000)
Parathion methyl	< 20	(< 5 000)
Phosalone	< 50	(< 5 000)
Phosmet	< 50	(< 5 000)
Phosphamidon	< 25	(< 5 000)
Profenofos	< 50	(< 5 000)
Prothoate	< 20	(< 5 000)
Pyridaphenthion	< 15	(< 5 000)
Pyrimiphos ethyl	< 20	(< 5 000)
Pyrimiphos methyl	< 15	(< 2 500)
Sulfotep	< 20	(< 5 000)
Temephos	< 15	(< 5 000)
Tetrachlorvinphos	< 30	(< 5 000)
Thiomethon	< 40	(< 5 000)
Triazophos	< 30	(< 5 000)
Trichlorfon	< 10	(< 5 000)
Trichloronate	< 25	(< 5 000)

SYNTHETIC PYRETHRINOIDS (µg / Kg)

ND ..... ND ..... ND

NOTES

Laboratoire Contrôle AQ
Le Responsable

2001/05/10

Le Responsable AQ

CERTIFICATE OF ANALYSIS FOR DRINKING WATER

ANALYTICAL DATA SUMMARY SHEETS

Huntingdon North Public Water Supply Zone

Population:- 47616 | 01-Jan-01 - 30-Jun-01 | Zone Code:- FW40 | Grid Ref:- TL245735

Parameter		PCV	Units	Number of samples		% samples contravening PCV	Concentration or Value (all samples)		
Ref	Name						Minimum	Mean	Maximum
A001	Colour	20	mg/l Pt/Co	6	R	0	< 1	< 1.37	2.7
A002	Turbidity	4	FTU	34	R	0	0.08	< 0.173	0.57
A003	Odour	3	Dil No	5	R	0	< 0	< 0	< 0
A03a	Odour - Nature	-	-	34		-	1	1	1
A03b	Odour - Intensity	-	-	34		-	1	1	1
A004	Taste	3	Dil No	5	R	0	< 0	< 0	< 0
A04a	Taste - Nature	-	-	34		-	1	1	1
A04b	Taste - Intensity	-	-	34		-	1	1	1
A005	Temperature	25	°C	61		0	2.7	10.8	20.3
A006	Hydrogen ion (pH)	5.5 - 9.5	pH	34	R	0	7.58	7.75	8.13
A007	Sulphate	250	mg/l	1		0	113	113	113
A008	Magnesium	50	mg/l	1		0	7.94	7.94	7.94
A009	Sodium	150	mg/l	1		0	32.1	32.1	32.1
A09a	Sodium 80*	150	mg/l	3		0	0	48.6	0
A010	Potassium	12 (15)	mg/l	10	X	0	5.87	6.69	7.3
A011	Dry Residues	1500	mg/l	1		0	532	532	532
A012	Nitrate	50	mg/l	13		0	14.4	22.8	25.1
A013	Nitrite	0.1	mg/l	13	I	30.77	< 0.003	< 0.058	0.191
A014	Ammonium	0.5	mg/l	6		0	0.161	0.175	0.201
A017	Total organic carbon	-	mg/l	1		-	3.78	3.78	3.78
A020	Surfactants	200	µg/l	1		0	< 18	< 18	< 18
A021	Aluminium	200	µg/l	6	R	0	< 3	< 7.4	25.3
A022	Iron	200	µg/l	8	R	0	< 14	< 14	< 14
A023	Manganese	50	µg/l	6	R	0	< 1	< 1	< 1
A024	Copper	3000	µg/l	1	R	0	40.6	40.6	40.6
A025	Zinc	5000	µg/l	1	R	0	< 14	< 14	< 14
A026	Phosphorus	2200	µg/l	3		0	483	720	1050
A027	Fluoride	1500	µg/l	1		0	253	253	253
B003	Cyanide	50	µg/l	1		0	< 5.3	< 5.3	< 5.3
B007	Lead	50	µg/l	1	R	0	< 3.2	< 3.2	< 3.2
P014	Chlorotoluron	0.1	µg/l	6		0	< 0.01	< 0.01	< 0.01
P032	Diuron	0.1	µg/l	6		0	< 0.01	< 0.01	< 0.01
P048	Isoproturon	0.1	µg/l	6		0	< 0.01	< 0.01	< 0.01
P051	Linuron	0.1	µg/l	6		0	< 0.01	< 0.01	< 0.01
P113	Monuron	0.1	µg/l	6		0	< 0.01	< 0.01	< 0.01
P006	Bentazone	0.1	µg/l	6		0	< 0.01	< 0.012	< 0.02
P026	Dichloroprop	0.1	µg/l	6		0	< 0.01	< 0.012	< 0.02
P054	MCPA	0.1	µg/l	6		0	< 0.01	< 0.012	< 0.02
P053	MCPP(Mecoprop)	0.1	µg/l	6		0	< 0.01	< 0.012	< 0.02
P004	Atrazine	0.1	µg/l	10		0	< 0.01	< 0.01	< 0.01
P070	Prometryne	0.1	µg/l	10		0	< 0.01	< 0.01	< 0.01
P066	Propazine	0.1	µg/l	10		0	< 0.01	< 0.01	< 0.01
P073	Simazine	0.1	µg/l	10		0	< 0.01	< 0.011	0.02
P077	Terbutryne	0.1	µg/l	10		0	< 0.01	< 0.01	< 0.01
P132	Trietazine	0.1	µg/l	10		0	< 0.01	< 0.01	< 0.01
B010	Pesticides - Total	0.5	µg/l	10		0	0	0.006	0.02
B011	PAH	0.2	µg/l	1	R	0	0	0	0
C001	Total Coliforms	0	No/dl	63		0	0	0	0
C002	Faecal Coliforms	0	No/dl	63		0	0	0	0
C003	Faecal Streptococci	0	No/dl	2		0	0	0	0
C008	Colony Count 1Day @ 37°C	-	No/ml	63		-	0	0.905	8
C012	Colony Count 7Day @ 22°C	-	No/ml	63		-	0	43.1	308
C010	Chlorine Total	-	mg/l	61		-	0.15	0.609	0.95

ANALYTICAL DATA SUMMARY SHEETS

Huntingdon North Public Water Supply Zone

Zone Code:- FW40
Grid Ref:- TL245735

Population:- 47616

01-Jan-01 - 30-Jun-01

Parameter Ref	Name	PCV	Units	Number of samples	% samples contravening PCV	Concentration or Value (all samples) Minimum	Mean	Maximum
D01a	Conductivity - M12	1500	µs/cm	34	0	741	752	767
D02a	Chloride - M12	400	mg/l	1	0	55	55	55
D03a	Calcium - M12	250	mg/l	1	0	128	128	128
D07a	Benzo 34 pyrene - M12	10	ng/l	1	0	3	3	3
D08a	Tetrachloromethane - M12	3	µg/l	6	0	0.078	0.089	0.1
D09a	Trichloroethene - M12	30	µg/l	6	0	0.308	0.355	0.4
D10a	Tetrachloroethene - M12	10	µg/l	6	0	0.235	0.268	0.3
E001	Hardness as Ca - Min	-	mg/l	1	-	158	158	158
E002	Alkalinity - HCO3 - Min	-	mg/l	1	-	253	253	253

Notes

PCV - Prescribed concentration or value
M12 - Rolling 12 month mean
M3 - Rolling 3 month mean
Min - PCV is a minimum only where the water is softened
U - Undertaking
X - Relaxation (relaxed value in brackets under PCV column)
R - Reduced sampling frequency
I - Increased sampling frequency
PAH - Polycyclic aromatic hydrocarbons

Sodium 80* - the 80th percentile of the last 3 years of sodium results